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1. The columns of the Journal are open for contributions dealing with veterinary science. Papers having a direct bearing on this subject are acceptable.

2. The Editorial Board accepts papers on the understanding that they are offered to the J.C.P.T. alone and reserves the right to reject contributions submitted for publication. During the period of the war it is essential that all articles shall be as concise as possible.

3. Citation of literature in the text should be accompanied by the year of publication. Authors' names (followed by year, publication, etc.) should be arranged alphabetically in a list at the end of the paper.

4. Papers for publication, exchange journals, etc., should be sent to Prof. T. J. Bosworth, "The Beeches," Streatley, Berks, or to any member of the Editorial Board.

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FOREWORD

FOUNDED in the year 1888 *by the late Sir John M'Fadyean, the Journal of Comparative Pathology and Therapeutics was issued continuously under his editorship until publication was suspended in March,* 1940. *Throughout this period the journal remained in his sole ownership. Sir John died in February,* 1941.

In 1938 *plans were mooted by him for the continuation of his journal whereby a small board of veterinary workers was invited by him to carry on the task of editorship in accord with his strictly expressed wishes. Since publication was allowed to lapse in the early uncertain phase of the present war, veterinary research workers have felt a growing need for the issue of a journal conforming in scope and standard with the Journal of Comparative Pathology and Therapeutics. The Trustees of Sir John's estate have already made public their desire,* Vet. Rec., 1942, *May 2nd,* 178, *that the journal should continue and that the task of publication should be delegated to the board of editors selected by him; ownership has been transferred by them to this board. Both as an act of devotion to his memory and to meet the prevailing demand for a medium of publication of this kind, the members of the board have felt it to be their duty to accept the responsibility for the re-issue of the journal, hoping that it is in their power to maintain the high standard upon which Sir John insisted during his lifetime, while at the same time eliciting the goodwill and confidence of the increasing number of workers engaged in the field of veterinary research who would desire to have their papers published in the journal.*

Since the journal first appeared in 1888, *veterinary science has expanded in many different directions and, in conformity with its late Editor's wishes, the members of the board will be happy to receive from authors, for consideration as to its suitability for publication, any paper which represents in their judgment a serious contribution to the advancement of knowledge within the now much expanded field. They need not, even if it were possible to do so, attempt to define the limitations of that field. Every endeavour will be made to deal with authors' papers promptly. A few rules for the guidance of contributors in the preparation of their papers are printed on the title page.*

CRYSTAL VIOLET VACCINE AGAINST SWINE FEVER A FIELD TRIAL

By

H. T. Matthews and T. M. Doyle

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A preliminary report (Doyle, 1942) has been published giving the results of immunity tests with crystal violet vaccine against swine fever carried out under laboratory conditions. It was shown that in the particular series of tests the vaccine protected pigs for at least ten months. In view of this result, it was decided to test it on a small scale under the varying conditions that obtain in the field.

The procedure followed was to purchase store pigs in the open market, vaccinate them at the laboratory and later move them to suitable field outbreaks of the disease. Three (W, Z, 22) different brews of vaccine were used. Each pig was given a dose of 10 c.c. subcutaneously and held at the laboratory sufficient time to allow for the establishment of immunity. The history of the experimental pigs was not known. Most of them appeared to be in normal health, but two small groups showed scurfy skin and coughed intermittently. They were bought immediately after weaning and were held for at least 14 days in isolation at the laboratory to guard against their being naturally infected with swine fever. In addition one or two pigs from each litter were inoculated with virus to verify their susceptibility. It has been the laboratory practice for many years to buy young pigs, either in the open market or from selected farms, and none has been encountered that has survived the inoculation of our test virus. A selection was made of naturally occurring outbreaks of the disease considered suitable for the trial based on factors such as willingness of owner to co-operate, distance from the laboratory, probability of stock remaining on the infected premises for a period long enough to observe events and suitability of premises for arranging close contact between sick and protected pigs. During the period of the trial, outbreaks happened to be particularly few, so that the standard of general suitability desired could not be maintained. In no sense was there, however, selection on factors which might have affected results in favour of the vaccine.

On receipt of information that a suitable outbreak had occurred, and sometimes before diagnosis was officially established, one or more vaccinated pigs were transported by van from the laboratory to the infected premises, and one or more sick pigs were brought back from the outbreak to the laboratory in the same van. These sick pigs (referred to as " test " pigs) were tested at the laboratory for the presence of virus.

The vaccinated pigs were placed in as intimate contact as could be arranged with sick pigs remaining on the infected premises and they were observed at least once weekly over a period of not less than

28 days. Owners were invited to expose the vaccinated pigs to infection by all available means. Housing in the same pen with sick pigs, or a succession of them, deliberate neglect of conditions of general hygiene, and such things as leaving the slashed carcases of dead pigs in the pen, were practised.

Results.—Thirty-six vaccinated pigs were exposed in 17 outbreaks. Twenty-nine came through the trial period without incident and thrived. The history of the remaining seven pigs was as follows :

Pig 1322 (Case 4) refused food on the fifth day of exposure, showed signs that suggested clinical swine fever, not severe, but it had recovered completely in eight days and thrived afterwards. This may be regarded as a partial breakdown of protection. This pig was severely tested as it was put in a pen with six clinically affected pigs, all of which subsequently died. Three other trial pigs exposed on the same premises but in different pens showed no reaction at all.

Pig 1597 (Case 12) failed to thrive. His companion, Pig 1598, under the same conditions, grew normally. No. 1597 was returned after 33 days to the laboratory and grew rapidly on meal rations. No. 1602 was then sent to the outbreak, replacing 1597, and also proved slow in development without showing any definite signs of illness. The failure of these pigs to thrive normally can probably be attributed to a combination of factors, including poor diet (watery swill), but unconnected with swine fever virus.

Pig 1828 (Case 16) died on the 14th day after a short, acute illness. Post-mortem examination showed an extensive haemorrhage of the stomach wall and deep haemorrhage of the kidneys.

Pig 1831 (on same outbreak) sickened at the same time as 1828, showed clinical signs suggestive of swine fever, was returned to the laboratory on 16th day and there recovered. No. 1828 may be regarded as a complete and 1831 as a partial breakdown of protection.

Pigs 1656 and 1657 (Case 17) refused food on the 10th day of exposure but recovered completely in three days, without apparent setback in condition. This is regarded as a partial breakdown.

Comment.—The trial was severe in the sense that the vaccinated pigs were involved in a journey, changed from meal diet on to swill in all cases, moved from clean comfortable conditions to those which, in some cases, were deliberately made uncomfortable. The exposure to infection with virus was intimate in all cases. The detailed record of events in the case papers are summarised in Table I, and it will be seen that the vaccinated pigs usually arrived on infected premises just after the peak of infection, but examination of the test pigs at the laboratory indicated (with one exception—Case 15) that virus was present on the premises in each case at the time of exposure of the vaccinated pigs : in addition, the deaths and illness among the control pigs on the premises, after the arrival of vaccinated pigs, is a fair index of the continued presence of virus. The trial extended from June 16th, 1941, to January 16th, 1942, embracing the extremes of heat and cold likely to be encountered in Great Britain. Allowing for war conditions, swill feeding, shortage of bedding and labour, difficulty of repair to buildings, universality of which inevitably lower the standard, the normal range of variation

in general hygiene was covered in the trial, but in most cases the standard was low. Variations in the pathogenicity of the virus were encountered. Tending in favour of the vaccine was the fact that the vaccinated pigs were strong stores when exposed.

Morbidity and mortality rates for the series of trial outbreaks were reckoned from the onset of the infection, not from the date of beginning of the trial period, which generally followed very closely

TABLE I

Case	*Initial Number*	*Events Relative to Introduction of Trial Pigs*															
		Deaths								*Sickness*							
		Weeks Prior				*Weeks Following*				*Weeks Prior*				*Weeks Following*			
		4	3	2	1	1	2	3	4	4	3	2	1	1	2	3	4
1	7	–	1	–	11	1	–	–	–	1	1	1	3	1	1	1	1
2	3	–	–	2	–	1	–	–	–	–	–	2	2	–	1	1	1
3	44	–	–	4	10	2	1	2	1	–	–	22	13	3	3	1	–
4	42	3	1	7	7	23	5	2	–	8	9	14	32	7	2	–	–
5	7	–	–	2	1	1	–	1	–	–	–	9	7	5	3	3	2
6	7	2	–	1	1	3	2	–	–	–	–	8	7	4	1	1	–
7	4	–	–	–	–	1	–	–	1	9	9	6	4	1	2	2	1
8	5	–	–	2	1	–	2	–	–	–	–	2	3	4	1	1	1
9	41	–	–	2	1	2	4	–	1	–	–	10	9	5	3	1	2
10	4	–	–	–	1	–	1	–	–	–	–	–	1	1	1	1	1
11	8	–	–	2	1	–	–	–	2	–	8	6	5	3	3	3	1
12	–	–	–	–	1	–	1	2	2	–	–	2	4	3	3	7	10
13	91	–	–	6	4	14	14	12	13	?	?	?	21	?	?	?	?
14	122	–	–	1	2	2	–	–	–	–	–	2	3	2	1	–	–
15	4	–	1	–	1	–	1	–	–	–	1	5	4	1	–	–	–
16	24	–	3	4	4	2	2	–	–	–	–	5	5	2	2	–	–
17	10	–	1	–	2	4	–	–	–	–	3	9	7	2	2	2	1

after the time of official diagnosis. These are summarised in Table II. The number of pigs recorded as remaining normal is not an accurate figure in the precise sense of resistance. It is the number which did not show any clinical sign of the disease for one reason or another. Many were subjected to serum treatment and many were so segregated from infected pigs as to make it probable that they were not

truly exposed to virus. It is not possible under the conditions obtaining in this trial to ascertain just how many of the pigs would have proved naturally resistant but, from our knowledge of the general conditions of management, an estimate has been made that,

TABLE II
MORBIDITY AND MORTALITY

Case	*Number Exposed at Onset of Outbreak*	*Number Slaughtered, healthy within 28 Days*	*Number moved on*	*Number Born*	*Number Regarded as Exposed to Infection*	*Died of S.F.*	*Recovered*	*Remained Normal*
1	29	4	—	—	25	20+[1]	1	3
2	4	–	1	—	5	4+[1]	—	—
3	72	13	—	—	59	20+[1]	—	38
4	62	—	—	—	62	49+[1]	2	10
5	11	—	—	—	11	6+[1]	3	1
6	14	3	—	—	11	8+[2]	1	—
7	13	4	—	—	9	8+[1]	—	—
8	8	—	—	—	8	6+[1]	1	—
9	63	19	—	—	44	11+[1]	3	29
10	5	—	1	—	6	3+[1]	1	1
11	11	2	—	—	9	5+[1]	2	1
12	35	14	—	—	21	11+[2]	4	4
13	102	—	—	23	125	63+[1]	—	61
14	122	19	—	—	101	5	3	93
15	6	—	—	—	6	3	2+[1]	—
16	55	34	—	—	21	18+[1]	+2	—
17	10	—	—	—	10	8+[1]	1	—
					533*	265 % 49·7	27 % 5·0	241* % 45·3

Note.—+ = test pigs moved to laboratory.
* For amendment of these figures, see text.

of those truly exposed and not artificially protected, about ten resisted. The degree of exposure of vaccinated pigs was certainly more intimate than would obtain in the case of these ten, but the comparison is the fairest possible in the circumstances. On this method of

calculation, the effective number of control pigs is reduced from 533 to 302, which gives the following :—

	Number Exposed to Infection	*Died of Swine Fever*	*Recovered from Swine Fever*	*Resisted Infection*
Control pigs ...	302	265 (87·8%)	27 (8·9%)	10 (3·3%)
Vaccinated pigs ...	36	1 (2·8%)	4 (11·1%)	31 (86·1%)

National mortality and morbidity rates in swine fever outbreaks against which the value of results of vaccination could be assessed have not been established, but an analysis of figures obtained from Ministry of Agriculture records, which are given in Table III, may be helpful.

TABLE III

ANALYSIS OF OUTBREAKS OF SWINE FEVER DURING 1938-39-40

	Great Britain	*England*	*Wales*	*Scotland*
Total number of outbreaks	9,256	8,784	262	210
Initial pig population on affected farms ...	504,991	475,562	9,279	20,150
Deduct pigs slaughtered for food or moved off	253,491	236,262	4,406	12,823
=	251,500	239,300	4,873	7,327
Add pigs born or moved on	57,280	54,700	560	2,020
=	308,780	294,000	5,433	9,347
Deduct deaths not from swine fever ...	2,953	2,795	34	124
Total pigs exposed to infection and observed at termination of outbreak	305,827	291,205	5,399	9,223
Total deaths from swine fever	217,964	207,356	4,462	6,146
Total survivors	87,863	83,849	937	3,077
	%	%	%	%
Probability of death from swine fever ...	71·2	71·2	82·6	66·6
Probability of survival	28·8	28·8	17·4	33·4

Summary and Conclusion.—Of 36 vaccinated pigs severely exposed in 17 naturally occurring outbreaks of swine fever, one died of acute swine fever, one became ill but recovered, three showed reactions of a minor character, and 31 resisted infection. Crystal violet vaccine appears to have afforded a high degree of protection in this series of cases.

ACKNOWLEDGMENT

We are much indebted to the Staff of the Animal Health Division, Ministry of Agriculture, in Kent, Surrey, Sussex East, Sussex West, Berkshire and Hampshire for their co-operation in this work.

REFERENCE

Doyle, T. M. (1942). *Vet. J.*, **98**, 51.

APPENDIX

CASE No. 1

Vaccinated Pigs.—1315 and 1316 moved to infected farm on 28th day after vaccination. Both remained normal.

Test Pig.—1441 died of S.F. two days after arrival at laboratory. Pig 1383, susceptible, in-contact to 1441 for 24 hours and then inoculated with spleen suspensions from 1441. Died of S.F. on the 16th day.

Control Pigs.—Twenty died, one recovered, three resisted infection. No serum given.

CASE No. 2

Vaccinated Pig.—1325 moved to infected farm 38 days after vaccination. Remained normal.

Test Pig.—1450 died of S.F. 32 days after arrival at laboratory. Pig 1392, susceptible in-contact to 1450, destroyed *in extremis*, 23rd day of S.F.

Control Pigs.—Five died of S.F., including an unvaccinated pig 1389 sent from laboratory to infected farm, sick 12th day and died 29th day. None recovered, none resisted infection.

CASE No. 3

Vaccinated Pigs.—1319, 1320, 1323 moved to infected farm on 30th day after vaccination. Remained normal.

Test Pig.—1448 died on 12th day of S.F. No. 1386 susceptible in-contact died on 22nd day of S.F.

Control Pigs.—Twenty died of S.F. None recovered, 38 resisted infection following the injection of serum.

CASE No. 4

Vaccinated Pigs.—1318, 1321, 1322, 1326 moved to infected farm on 44th day after vaccination. No. 1322 was put in contact with six clinically affected animals, all of which subsequently died. It became sick after four days' contact; anorexia, hemiplegia, loss of weight, clinically S.F.; fully recovered eight days later and thrived afterwards. The other three vaccinated pigs remained healthy.

Test Pig.—1470 died on eighth day of S.F.; 1360 susceptible in-contact, died on 33rd day of S.F.

Control Pigs.—Forty-nine died of S.F. Two recovered, ten resisted infection. (Six pigs effectively isolated.)

CASE No. 5

Vaccinated Pigs.—1442, 1443 moved to infected farm on 17th day after vaccination. Both remained normal.

Test Pig.—1471 died on arrival at laboratory of S.F. No. 1465 inoculated with 1 c.c. spleen suspension from 1471, died on 48th day of S.F. No. 1466, an in-contact control to 1465, survived. Tested later with virus, it resisted infection. It is probable that 1466 acquired immunity from contact with 1465.

Control Pigs.—Six died of S.F. Three recovered, one resisted infection.

CASE No. 6

Vaccinated Pigs.—1456 and 1459 moved to infected farm on 67th day after vaccination. Both remained healthy.

Test Pigs.—1627 and 1628 died on fourth and eighth day respectively of S.F. No. 1590 in-contact died on 22nd day of S.F.

Control Pigs.—Eight died, one recovered, none resisted infection.

CASE No. 7

Vaccinated Pigs.—1453, 1461, moved to infected farm on 62nd day after vaccination. Both remained normal.

Test Pigs.—1604 died on 18th day of S.F. 1578 susceptible in-contact died 25th day of S.F.

Control Pigs.—Eight died, none recovered, none resisted infection.

CASE No. 8

Vaccinated Pigs.—1457 and 1460 moved to infected farm on 67th day after vaccination. Both remained normal.

Test Pigs.—1629 died on fourth day of S.F. (this pig had received serum on farm); 1589, in-contact, died on 25th day of S.F.

Control Pigs.—Six died, one recovered, none resisted infection. Serum injected.

CASE No. 9

Vaccinated Pigs.—1601, 1603 moved to infected farm on 21st day after vaccination. Both remained normal.

Test Pigs.—1659 died on night of arrival at laboratory of S.F. No. 1616, inoculated with spleen suspension from 1659, became sick on 27th day and showed anorexia, diarrhoea, and unsteady gait. Made complete recovery in 24 days and was tested for immunity by inoculation of virus 49 days after the original inoculation. Resisted infection. Two control pigs inoculated with the same virus died on the 10th and 14th day, respectively.

Control Pigs.—Eleven died, three recovered, 29 resisted infection. Serum administered.

CASE No. 10

Vaccinated Pigs.—1472 and 1473 moved to infected farm on 40th day after vaccination. Both remained normal.

Test Pigs.—1594 died on 11th day of S.F. No. 1571, in-contact, died 16th day of S.F.

Control Pigs.—1570, susceptible pig taken from laboratory to farm became sick on 14th day, died 39th day of S.F. Including this pig, three died, one recovered, and one resisted infection.

CASE No. 11

Vaccinated Pigs.—1458 and 1595 moved to infected farm on 81st day after vaccination. Both remained normal.

Test Pigs.—1636, died 13th day of S.F. No. 1605, in-contact, died 17th day of S.F.

Control Pigs.—Five died, two recovered, and one resisted infection.

CASE No. 12

Vaccinated Pigs.—1597, 1598 moved to infected farm on 23rd day after vaccination. No. 1598 remained normal; No. 1597 failed to thrive; no definite clinical signs; returned to laboratory, put on good rations, grew normally and was later sold for food. No. 1602, replacing 1597, remained normal, but grew slowly. The diet given to these pigs was exceptionally bad, mainly thin watery swill.

Test Pigs.—No. 1673 died on second day of S.F. No. 1617, susceptible in-contact, remained healthy. Inoculated later with virus, it resisted infection. This pig had presumably acquired immunity by contact. No. 1807, a sick pig from farm, destroyed on arrival at laboratory. P.M. showed one small ulcer in caecum. No. 1728, inoculated with spleen suspension from 1807, died on 23rd day of S.F. No. 1658 (vaccinated pig) in contact for

six days with 1728, and inoculated with spleen suspension from 1728, died 15 days after first contact and nine days after inoculation.

P.M.—Petechiae on skin ; body lymphatic glands enlarged and haemorrhagic ; petechiae on kidneys.

S. cholérae suis was isolated in pure culture from the spleen. The lesions exhibited might have resulted either from acute swine fever or *S. cholerae suis* bacteraemia.

Pig 1882 (given 40 c.c. swine fever immune serum) and an untreated pig, 1888, were in-contact with 1658 for three days before it died. Both remained normal. Inoculated with virus 35 days later, both died. Pig 1886, given 1 c.c. spleen suspension from 1658, remained normal. Inoculated with virus 30 days later ; it died.

Conclusion.—Inoculation confirmed the presence of swine fever on the farm. It would appear reasonable to conclude from the evidence that vaccinated pig 1658 died of *S. cholerae suis* bacteraemia and not from swine fever.

Control Pigs.—Eleven died of S.F., four recovered, four resisted infection.

Case No. 13

Vaccinated Pigs.—1596, 1600 moved to infected farm on 56th day after vaccination. Both remained normal.

Test Pigs.—1769 died on first day of S.F. No. 1720, in-contact for 24 hours and inoculated with spleen suspension from 1769, died on eleventh day of S.F.

Control Pigs.—Sixty-three died of S.F. Sixty-one treated with serum resisted infection.

Case No. 14

Vaccinated Pigs.—1653, 1655 moved to infected farm on 20th day after vaccination. Both remained normal.

Test Pigs.—No live pig submitted. Spleen and liver from two sick pigs inoculated into Pig 1825, which died on 26th day of S.F.

Control Pigs.—Five died, three recovered, 96 resisted infection ; 64 of the in-contact pigs were treated with serum ; 22 pigs effectively isolated on separate premises.

Case No. 15

Vaccinated Pig.—1654 moved to infected farm on 25th day after vaccination. Remained normal.

Test Pigs.—1871 (had been treated with serum on farm) apparently normal up to 18th day. Inoculated with virus, remained normal. No. 1827, in-contact, remained normal. Inoculated with virus, died on 7th day of S.F.

Control Pigs.—Three died, two recovered. Swine fever probably existed in this herd, but on account of the use of immune serum its presence could not be confirmed.

Case No. 16

Vaccinated Pigs.—1828 and 1831 moved to infected farm on 19th day after vaccination. 1828 died 14 days later. P.M. : haemorrhages stomach wall, deep haemorrhages kidneys, slight enteritis and pneumonia. ? S.F. : Pig 1932 inoculated with a mixed suspension of bone marrow, liver and spleen from 1828 showed slight reaction. Inoculated with virus 49 days later, remained normal.

Pig 1831 (vaccinated) returned to laboratory sick, recovered in a few days ; inoculated with virus 33 days later remained normal. No. 1626 in-contact for 33 days with 1831, remained normal. Inoculated with virus,

no reaction. Presumably had acquired immunity from contact with 1831. Two small store pigs, 1962 and 1963, were also sent from the farm to the laboratory. No. 1963 died 12 days later of necrotic enteritis (*S. cholerae suis*). No. 1962, which had been in-contact with 1831, was later inoculated with virus and remained normal.

Test Pigs.—No. 1940 sick, recovered in about ten days. Inoculated with virus, died on 24th day of S.F. No. 1862, susceptible in-contact with 1940, remained normal. Inoculated with virus, died on sixth day of S.F. The evidence here indicates that the sick pig, 1940, received from the outbreak, was not affected with S.F.

Control Pigs.—Eighteen died, two recovered (1940, 1962). None resisted infection. There was considerable circumstantial evidence that swine fever virus was the main cause of this outbreak, but the laboratory tests suggested that there was in addition some infection with *S. cholerae suis* (*e.g.*, Nos. 1963 and 1940). Vaccinated pig 1828 must be regarded as having died of swine fever ; it is possible that the rapid course of the disease in this animal may have resulted from a dual infection with swine fever virus and *S. cholerae suis*. The results of the biological tests suggested the presence of a low grade virus as certain pigs (1626, 1932, 1962) in-contact with the infection acquired immunity without exhibiting symptoms and later resisted the inoculation of a certain killing virus. There was no reason to doubt the original susceptibility of these animals.

Case No. 17

Vaccinated Pigs.—1656, 1657, moved to infected farm on 44th day after vaccination. On tenth day, dull, anorexia, discoloured ears, one coughed repeatedly, no diarrhoea, recovered in three days and thrived afterwards.

Test Pig.—1939, died one day of S.F. No. 1908, susceptible in-contact, died 38th day of S.F. No. 1726 (vaccinated pig) in-contact, remained healthy.

Control Pigs.—Eight died, one recovered, none resisted infection.

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A GUIDE TO THE INTERNAL STRUCTURE OF THE MEDULLA OBLONGATA OF THE SHEEP

By

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INTRODUCTION

In the investigation of diseases of the central nervous system, it is essential to examine the medulla oblongata. In animals the internal structure of this part of the brain stem is complex, and the identification of individual structures by analogy with man is difficult and confusing. There is, however, a close resemblance between the medulla of the sheep and that of the other domesticated mammals. Literature on the subject is sparse, in spite of the fact that the sheep's brain is very frequently used for demonstration purposes in classes of biology, and, therefore, we feel that a simple guide to the internal structure of the medulla oblongata of the sheep will be helpful to anatomists and comparative pathologists.

To define the precise limits of the medulla oblongata is not easy, but it is convenient to consider the posterior limit as that level at which the decussation of the cortico-spinal (pyramidal) tracts is complete, and the anterior limit as being just rostral to the facial genu. Neither the posterior limit nor the anterior is sharply demarcated, and this fact has been emphasised (unconsciously, perhaps) by Sisson (1938), who depicts the superior olive in a section through the anterior extremity of the medulla of the horse, but describes this structure under a general description of the pons Varolii. Kappers, Huber and Crosby (1936) avoid the difficulty by considering the medulla and pons together as a single entity. Macroscopically, the anterior limit of the medulla oblongata may be determined as the anterior border of the trapezoid body which, in the sheep, takes the form of a well-marked band of transverse fibres caudal to the broader band formed by the pontine fibres.

Textbooks of human anatomy depict the pyramidal decussation as a crossing of broad, dense fibre bands. King (1911) has described the pyramidal tracts of the sheep's brain as small and as decussating in the lower medulla and first cervical segment of the spinal cord. Most of the decussating fibres run upwards along the line of the median raphé, and are most easily demonstrated by the use of a myelin sheath stain. Followed rostrally to the level of the hypoglossal nucleus, the motor decussation becomes progressively less obvious, but at this level fibres of the sensory decussation (not at any time well marked) may be identified.

Our method of examination was by serial sections, and we would suggest that to identify any nucleus with certainty it would be helpful to cut two or three sections at, say, 20 sections apart. By cutting more than one section confusion of groups of cells of the reticular

formation with true nuclei is obviated. The majority of nuclei begin as one or two cells, increase to a maximum, and then become smaller again as they are followed rostrally. The notable exception to this rule is the nucleus ambiguus which appears, disappears, and appears again throughout its length. Finally, it might be mentioned that one seldom cuts a section exactly at right angles to the long axis of the medulla, and that in consequence the halves are seldom bilaterally symmetrical; but here again the examination of more than one section prevents confusion.

NUCLEI OF THE MEDULLA

It is realised that the size of a cell body as seen under the microscope depends to some extent upon the plane of section, but for descriptive purposes cells of similar size to the motor cells as seen in the sixth, seventh and twelfth cranial nuclei have been designated " large "; cells less than half this size have been called " small." Other sizes are comparative.

Abducens.—This nucleus, composed of large cells of motor type, is found in the rostral part of the medulla. Issuing fibres of the sixth cranial nerve run ventro-laterally from its medial border, and the nucleus dwindles rapidly as the facial genu curves round it anteriorly.

Accessory.—Large cells composing this nucleus continue into the medulla along the line of the ventral horn cells of the spinal cord, spreading somewhat more dorsally as the grey matter of the medulla broadens. Romanes (1940) has demonstrated that in the sheep these cells lie in the same cell-column as the nucleus ambiguus, but that there does not appear to be any continuity between these two nuclei. The cells of the accessory nucleus do not form a well-marked group in the medulla, and become confused with cells of the reticular formation and of the supraspinal nucleus.

Ambiguus.—Cells comprising this nucleus are large and possess long, sinuous dendrons which give to the nucleus itself a " wind-swept " appearance. The nucleus extends forwards from the posterior region of the medulla, and in some medullae is still visible at the level of the beginning of the facial nucleus. Typically, the nucleus ambiguus is composed of seven or eight cells closely related to each other and lying in the ventro-lateral reticular formation; but in some sections, throughout its length, the nucleus may be represented by a single cell—or may even be invisible. When only one or two cells of the nucleus ambiguus are present, it is difficult to differentiate them with certainty from adjacent cells of the reticular formation.

Cochlear Nuclei

Dorsal Cochlear.—This nucleus, which is not well defined, is composed of medium-sized cells which give rise to the striae medullares acousticae. These striae form an obvious band of fibres as they curve round the restiform body in the anterior third of the medulla.

Ventral Cochlear.—Rounded neurons of rather less than medium size found dorso-lateral (more rostrally, ventro-lateral) to the restiform body, and lying above, below, and among the fibres of the eighth cranial nerve, constitute the ventral cochlear nucleus, which extends forwards from about the middle of the anterior third of the medulla to the caudal border of the pons. This nucleus, together with the dorsal cochlear nucleus, is liable to be torn away with the overlying cerebellum.

Dorsal Motor Vagus.—From a level a short distance anterior to the posterior limit of the medulla, this nucleus, situated dorso-lateral to the central canal, can be traced forwards to a level beyond that at which the hypoglossal nucleus disappears. Macroscopically, the anterior limit of the nucleus may be determined as the caudal border of the facial tubercle. The dorsal motor vagus nucleus and the hypoglossal nucleus are closely related throughout their length, but the former is always the more dorsal. Most of the component cells of the dorsal motor vagus nucleus are of a distinctive rounded shape and appear as if they had been " combed out " in a direction at right angles to the median raphé. The cells are somewhat smaller than those of the hypoglossal nucleus, but larger than cells described as medium sized. At the level of the calamus scriptorius, and for some distance more anteriorly, the dorsal motor vagus nucleus lies beneath the ependymal and neuroglial lining of the fourth ventricle, but further forward still sinks away from the ventricle floor and finally disappears. Constituent fibres of the tenth cranial nerve can be seen leaving this nucleus throughout the greater part of its length.

Facial.—The large, well-defined facial nucleus extends through the anterior third of the medulla, but disappears before the caudal border of the pons is reached. Its component cells are large and are grouped to form a rough circle in the ventro-lateral reticular formation. One or two sections through the caudal end of the facial nucleus may show a remnant of the ambiguus nucleus, which occupies a more dorsal and more lateral position. In some sections it seems possible to divide the facial nucleus into four groups of cells. Of these, the middle contains the largest cells, and that group nearest to the median raphé is composed of cells which are smaller than those of the other groups. Macroscopically, a rounded protuberance on the ventro-lateral surface of the medulla corresponds with the underlying facial nucleus (see later, under " Discussion ").

Gracilis.—This nucleus, visible in sections through the posterior region of the medulla, is composed of small, inconspicuous cells scattered over a roughly oval area of grey matter which has its long axis parallel to and close to the mid-line, above the central canal. The nucleus is no longer recognisable when the level of the calamus scriptorius is reached. As the nucleus increases in size rostrally, the elevation due to the fasciculus gracilis disappears.

Hypoglossal.—The medium sized and large, typically motor cells of this nucleus first appear ventro-lateral to the central canal about

the middle of the posterior third of the medulla. Traced rostrally, the nucleus increases in size, but is always easily differentiated from the dorsal motor vagus which lies above it. At the level of the calamus scriptorius the hypoglossal nucleus forms part of the floor of the fourth ventricle, but more anteriorly it sinks into the grey matter and finally disappears before the anterior limit of the middle third of the medulla is reached. Towards its rostral extremity the hypoglossal nucleus appears to be composed of both medium and large cells, the former comprising the more medial part of the nucleus. Issuing fibres of the twelfth cranial nerve, running ventrally and somewhat laterally from the nucleus, are well defined in sections cut through the middle third of the medulla.

Inferior Olive.—Several cell-groups, arranged on either side of the median raphé and separated from each other by fibre whorls, combine to form the inferior olivary body which can be traced from about the middle of the posterior third of the medulla to the anterior end of the middle third. In sections stained to demonstrate myelin sheaths the inferior olive forms an easily recognisable structure, but when nerve-fibres are only faintly stained (*e.g.*, by the haematoxylin and eosin method) the exact relationship of cell-groups to fibres and to each other is not at all obvious. The shape of the inferior olivary body as a whole varies throughout its length, but its small, rounded neurons are constant in type, if not in position.

Inferior Salivary.—Just rostral to the anterior extremity of the facial nucleus, and in a slightly more dorsal position, a small group of cells similar to those of the ambiguus nucleus appears suddenly, persists through a few sections, and then disappears again with equal abruptness. This cell-group corresponds in size and position to the inferior salivary nucleus described in the dog.

Intercalatus.—As the central canal opens out rostrally into the fourth ventricle, both the hypoglossal nucleus and the dorsal motor vagus nucleus lie close beneath the floor of the ventricle. In a triangular area between these two nuclei and the ventricular floor a few medium-sized cells occur and may be distinguished as the nucleus intercalatus. Rostrally this nucleus merges with the paramedianus dorsalis and praepositus nuclei.

Interfascicularis.—At various levels of the medulla in which fibres of the hypoglossal nerve are well-marked, tightly packed groups of small and medium-sized cells are seen among the fibre-bundles. These cell-groups constitute the nuclei interfascicularis hypoglossi.

Lateral Cuneatus.—This well-defined nucleus is visible in sections cut through the middle third of the medulla, and appears in a position lateral and dorsal to that of the cuneate nucleus. The component cells are of medium size and numerous. Beyond the calamus scriptorius the lateral cuneate nucleus is displaced laterally by the developing medial vestibular nucleus, and finally disappears a short distance rostral to the level of the beginning of the facial nucleus.

Paramedianus Dorsalis.—A short distance anterior to the calamus

scriptorius, medium-sized cells appear between the hypoglossal nucleus ventro-laterally and the floor of the fourth ventricle dorsally (medial to the nucleus intercalatus). These cells have been described as the nucleus paramedianus dorsalis. It is difficult to define the precise limits of this nucleus and of the nuclei intercalatus and praepositus, all of which merge into each other and all of which are made up of neurons of very similar shape and size.

Praepositus.—The inter-relations of this nucleus have been mentioned under " paramedianus dorsalis " and " intercalatus." Towards the anterior extremity of the medulla the cells of the nucleus praepositus become smaller, merge gradually with those of the medial vestibular nucleus, and disappear before the abducens nucleus appears.

Retrofacial.—A few large cells situated ventro-medially to the nucleus ambiguus can be followed rostrally through a small number of sections just posterior to the beginning of the facial nucleus. It is not easy to say with certainty that these cells do actually constitute a separate nucleus, and that they are other than cells of the ventro-lateral reticular formation ; but they conform to descriptions of the retrofacial nucleus in man.

Roller.—In some sections a small collection of medium-sized cells is visible just ventral to the hypoglossal nucleus on the course of the emerging fibres of the hypoglossal nerve at a level a short distance rostral to the calamus scriptorius. This cell group constitutes the nucleus of Roller.

Solitarius.—The solitary tract, lateral to and close to the dorsal motor vagus nucleus, is visible from about the level of the calamus scriptorius to the level of the beginning of the lateral vestibular nucleus. The nucleus of this tract is represented posteriorly by a few small cells scattered round its borders ; more rostrally these cells increase in number, but few sections show the nucleus as a well-defined entity. More rostrally still the cells of the nucleus of the solitary tract become confused with the very similar cells of the spinal vestibular nucleus, so that it is difficult to say precisely where the one nucleus ends and the other begins.

Spinal Tract of the Trigeminal.—The spinal tract of the trigeminal nerve forms a well-defined landmark throughout the medulla, and its presence causes a bulging on the surface of the medulla—dorso-lateral in position posteriorly, lateral about the middle of the medulla, and ventro-lateral anteriorly. The small cells of its nucleus are found along its medial border.

Supraspinal.—This nucleus belongs to the posterior part of the medulla, in the region of the pyramidal decussation, and is not well marked. It is composed of a few large cells (a rostral continuation of the ventral horn cells of the spinal cord) scattered in the ventro-medial grey matter and frequently difficult to separate with certainty from cells of the accessory nucleus.

Superior Olive.—First traces of the superior olivary body are seen rostral to the middle of the anterior third of the medulla. When well developed this structure is composed of a triangular nucleus of medium-sized, rounded cells related laterally to two glial masses surrounded by fibre-whorls, with occasional rounded neurons among the fibre-bands. The superior olivary body lies in the ventro-lateral grey matter just dorsal to the trapezoid fibres, and can be traced rostrally into the pons.

Superior Salivary.—This nucleus is very similar in size and position to the inferior salivary nucleus, except that it is shorter and appears at a slightly more rostral level. It begins and ends abruptly, and is composed of cells similar to those of the ambiguus nucleus.

VESTIBULAR NUCLEI

Lateral Vestibular.—Very large cells which first appear about the middle of the anterior third of the medulla, ventro-medial to the restiform body, make up the lateral vestibular nucleus which can be followed rostrally as far as the caudal border of the pons.

Medial Vestibular.—When well developed, this nucleus occupies an area bounded by the dorsal surface of the medulla dorsally, the fourth ventricle medially, the dorsal motor vagus nucleus and the tractus solitarius ventrally, and by the lateral cuneate nucleus (or, more rostrally, the lateral vestibular nucleus) laterally. The component cells are numerous and of medium size. Towards the anterior extremity of the anterior third of the medulla this nucleus merges imperceptibly with the smaller cells of the superior vestibular nucleus.

Spinal Vestibular.—The cells composing this nucleus tend to be spindle-shaped, and their confusion with the anterior cells of the solitarius nucleus has been mentioned. It was considered possible to trace them anteriorly (ventro-lateral to the lateral vestibular nucleus) to the caudal border of the pons.

Superior Vestibular.—Followed rostrally, the medial vestibular nucleus decreases in size and finally shades into the superior vestibular nucleus, the cells of which are smaller, less numerous, and can be traced forwards into the pons.

Nuclei of the Reticular Formation.—That part of the medulla not composed of fibre-tracts or of nuclei as described above, is filled in by a network of grey matter and fine fibre-bundles, the reticular formation. Nerve cells of all shapes and sizes are scattered throughout this network, but here and there groups of neurons occur which do resemble true nuclei. Such cell-groups may appear suddenly, persist through several sections in series, and then disappear just as suddenly —and may sometimes be mistaken for nuclei of the cranial nerves.

Special reference may be made to three groups of reticular cells, which are themselves subdivisible into smaller groups :—

1. The nuclei of the raphé, composed of medium, large and very large cells, and best developed from about the middle of the medulla to the middle of its anterior third.

2. Magnocellular part of the reticular formation, made up of large and very large cells found in the ventro-medial reticular formation of the anterior third of the medulla, and covering a roughly triangular area divided symmetrically by the median raphé.

3. Infratrigeminal part of the lateral reticular formation (especially well marked through the anterior third of the medulla), composed of a group of medium-sized cells which are closely related to the tip of the descending tract of the trigeminal nerve. In some sections this cell-group may be confused with the ambiguus nucleus—but is always more ventral and more lateral in position.

Roller's nucleus and the nuclei interfascicularis hypoglossi have, in man, been described as nuclei of the reticular formation (Ranson), but in this paper have been mentioned separately.

Area Postrema.—This highly vascular glial network projects into the fourth ventricle near its caudal extremity, and is visible through a number of sections cut in this region.

DISCUSSION

In the general description of the facial nucleus given above, it has been mentioned that the position of this nucleus is marked macroscopically by a rounded prominence on the ventro-lateral surface of the medulla. This prominence is situated just caudal to the transverse ridge of the trapezoid fibres, and just lateral to the less obvious ridge produced by the fibres of the tractus lateralis minor. Wilkie (1937) and Ranson (1941) state that in the sheep this rounded elevation is due to the presence of the subjacent inferior olive, but we suggest that it corresponds with neither the inferior olive nor with the superior. Indeed, it has been found that sections cut through the entire length of this macroscopical protuberance show neither of the olivary bodies, but do show the facial nucleus lying directly underneath—and reaching its maximum development in sections cut through the middle of the protuberance. Therefore it seems obvious that this elevation corresponds with the facial tubercle described in the horse.

In man the emerging fibres of the hypoglossal nerve pass over the medial area of the olivary complex; in contrast to this it has been noted that in the medulla of the sheep fibres of this nerve pass from the brain stem lateral to the inferior olivary body, except in a few sections in which the inferior olivary body attains maximum development.

SUMMARY

The positions of the principal nuclei of the medulla oblongata of the sheep are described. The appearance of a protuberance on the ventro-lateral surface of the medulla corresponding with the underlying facial nucleus is noted, and commented upon. The position of the issuing fibres of the twelfth cranial nerve is contrasted with the position of these fibres in the medulla of man.

ACKNOWLEDGMENTS

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LEGEND FOR FIG. 1

SECTION No. 1.—This section is cut near the caudal extremity of the medulla. The rostral continuation of the ventral horn cells is shown as a narrow rectangle. The accessory nucleus has already disappeared, but the decussation of the pyramidal tracts is still visible and is represented by a collection of short curved lines spreading from the median raphé. The curved cross-section of the spinal trigeminal tract is outlined, and this persists throughout the medulla.

SECTION No. 2.—The inferior olive is now apparent, and the emerging fibres of the hypoglossal nerve can be distinguished.

SECTION No. 3.—The inferior olive persists. The dorsal motor nucleus of the vagus, capped by the area postrema, sends forth fibres that pass laterally to cross the spinal trigeminal tract. Dots interrupting the issuing fibres of the hypoglossal nerve mark the position of one of the nuclei interfascicularis hypoglossi.

SECTION No. 4.—The nucleus of the solitary tract is indistinct, but its position in this section is indicated by dots. The inferior olive is still apparent.

SECTION No. 5.—The restiform body is marked R.B. Dots indicate the position of the nucleus paramedianus dorsalis, and fibres from the dorsal motor nucleus of the vagus and to the solitary tract are shown crossing the spinal trigeminal tract.

SECTION No. 6.—The restiform body is shown capped by a collection of fibres, the striae medullares acousticae. Lying among these fibres are cells which constitute the dorsal cochlear nucleus. Dots beneath the floor of the fourth ventricle indicate the position of the nucleus praepositus.

SECTION No. 7.—The nucleus praepositus persists. The facial nucleus is terminating, and fibres from this nucleus are now gathered together to form a circular band that passes over the nucleus of the abducens nerve as the facial genu—which is represented by a circular outline containing crosses. At the ventral tip of the spinal trigeminal tract the infratrigeminal part of the lateral reticular formation is represented by dots. The fibres of the acoustic nerve enter laterally and split into dorsal and ventral groups, while association fibres connected with the nuclei of this nerve pass along the ventral margin of the medulla as the trapezoid body and are said to send fibres to the superior olives. The dorsal and ventral cochlear nuclei have been torn from this section with the overlying cerebellum.

SECTION No. 8.—This is the most rostral section of the series. Emerging fibres of the abducens nerve are visible, and fibres of the facial nerve (having completed their twist round the abducens nucleus rostral to this section) are seen emerging laterally.

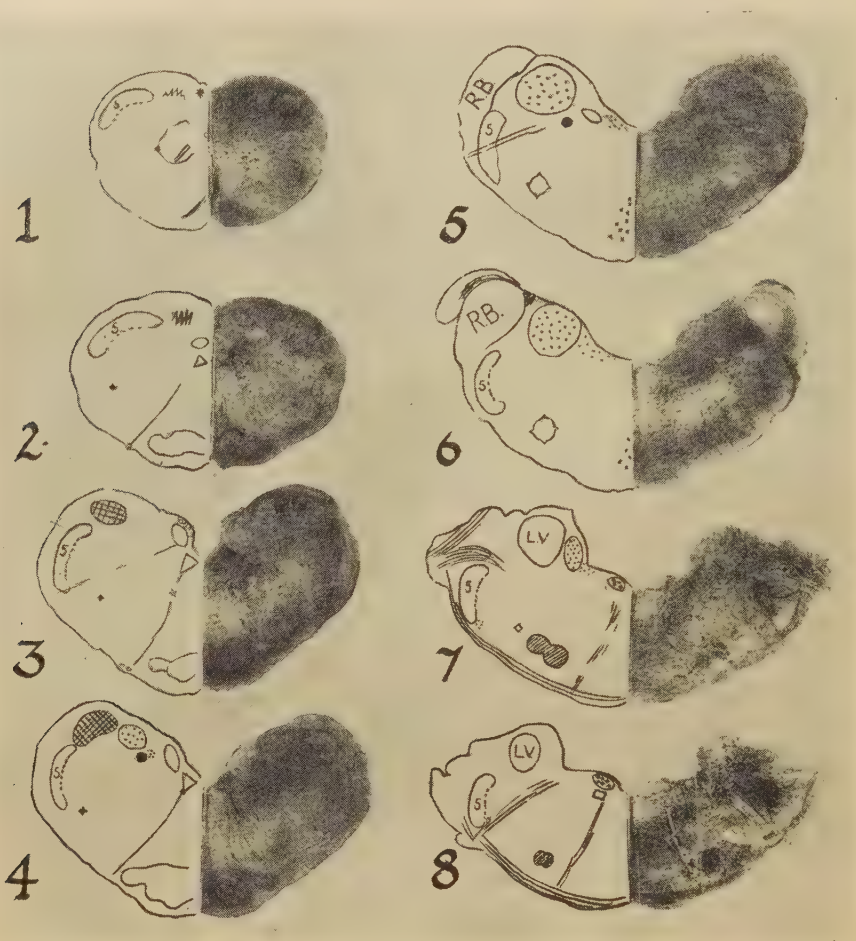

FIG. 1.—Series of transverse sections of the medulla.

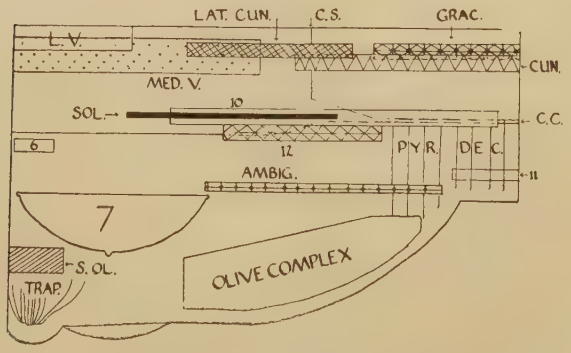

FIG. 2. Diagram of side view of medulla. Linear dimensions of the main muclei are shown.
C.S.: calamus scriptorius.
C.C.: central canal.
PYR. DEC.: pyramidal decussation.

KEY FOR FIG. 1 AND FIG. 2

Fig. 1	*Fig.* 2	*Main Features*
△	12	Hypoglossal nucleus.
□	6	Abducens nucleus.
⬭	10	Dorsal motor vagus nucleus.
◇	7	Facial nucleus.
	MED. V.	Medial vestibular nucleus.
	S. OL.	Superior olive.
	LAT. CUN.	Lateral cuneate nucleus.
●	SOL.	Solitarius tract and nucleus.
✦	AMBIG.	Ambiguus nucleus.
× × ×	—	Magnocellular nucleus.
✳	GRAC.	Gracilis nucleus.
	CUN.	Cuneatus nucleus.
	OLIVE COMPLEX	Inferior olivary body.
	—	Rostral continuation of ventral horn cells.
	—	Area postrema.
L.V.	L.V.	Lateral vestibular nucleus.
R.B.	—	Restiform body.
5	—	Spinal trigeminal tract and nucleus.
	—	Other small nuclei (see Legend.)

INFECTION OF LABORATORY ANIMALS WITH *MYCOBACTERIUM JOHNEI*

By

JOHN FRANCIS

INTRODUCTION

THE organisms causing Johne's disease were first observed by Johne and Frothingham in 1895, and in 1906 Bernard Bang showed that this disease and tuberculosis were distinct. F. W. Twort first cultivated *Myco. johnei* in 1910, but experimental work on the disease has been greatly hampered because attempts to infect laboratory animals have either failed or have not been consistently successful.

Twort and Ingram (1913) failed to infect pigeons, fowls, guinea pigs, mice and rats, although large doses of *Myco. johnei* culture were used and the animals were killed at periods varying from two months to two years after treatment. Meyer (1913) obtained similar negative results. Twort, C. C. (1914) gave eleven rabbits large intravenous doses of *Myco. johnei* grown in glycerin beef broth. Five of the eleven inoculated rabbits developed a light infection of the terminal 12 inches of the small intestine. The abdominal and thoracic lymphatic glands, the liver and the spleen contained numerous bacilli. Guinea pigs appeared to be more resistant than rabbits but five out of 28 mice inoculated intraperitoneally developed the disease. The alimentary tract was heavily infected from the stomach to the rectum and organisms were also found in the abdominal and thoracic lymphatic glands, the liver, and the spleen. Andersen (1922) gave rabbits injections of infected goat mesenteric lymphatic gland, infected bovine intestinal mucosa and pure cultures of *Myco. johnei* culture. In all, 16 rabbits were injected by various routes; two became severely and four moderately infected. Attempts to carry out passage experiments or to infect a fowl and a guinea pig all failed.

Following the observation of Twort and Ingram (1913), and Boquet (1925), that rabbits and rats receiving intraperitoneal injection of *Myco. johnei* culture developed nodules in the mesentery, Hagan and Mansfield (1930) repeated the experiments in guinea pigs. They found bacilli in the mesenteric nodules four to six months after inoculation but they were unable to culture these organisms: they also produced similar lesions by the use of heat killed *Myco. johnei* and *Myco. tuberculosis* organisms. Hagan and Levine (1932) showed that lesions could be produced with various saprophytic acid fast bacilli and killed cultures of Johne or tubercle bacilli suspended in saline; suspension in liquid paraffin greatly increased the subsequent changes. Mohler (1939) claimed to have infected laboratory animals by injecting, intraperitoneally, suspensions of *Myco. johnei* in liquid paraffin. Many of the injected animals died and Mohler concluded that the liquid paraffin acted as a culture medium in which the organisms multiplied until they overcame normal tissue resistance. He also claimed to have infected laboratory animals by similar injections of infected bovine material and to have passaged the infection through a series of animals. These observations were not confirmed by Sahai (1940 and 1941) or by Wilson Taylor (1940). Van Heelsbergen (1931) failed to infect hens, and Dunkin (1938) failed to infect hamsters.

Meyer (1913) carried out inoculation and feeding experiments in cattle and concluded that only calves in the first three or four months of life were

susceptible and that contact infection probably occurred in the first few days of life. Le Sueur (cit. Twort and Ingram, 1913) concluded from clinical observations that adult cattle were resistant. Hagan (1938) has produced convincing evidence that a marked age resistance develops in calves within four months of birth. McEwen (1939) has shown that sheep also develop an age resistance.

Because of this evidence that even naturally susceptible animals become relatively immune at an early age, and because there was no evidence that young laboratory animals had ever been used in experimental work, the writer attempted to establish infection by inoculating very young laboratory animals.

EXPERIMENTAL

A primary culture which had been isolated from a bovine mesenteric lymphatic gland was obtained from the Royal Veterinary College. This culture grew typically on the egg medium prepared as described by Dunkin (1928), except that no gentian violet was added, but failed to grow on plain egg medium. Fourteen weeks after isolation subcultures were made on three slopes of Dunkin's egg medium and they were incubated for 40 days at 37° C. Fifty milligrammes of moist bacilli were obtained from two of the slopes and emulsified in saline. This material was used to inoculate mice and hamsters.

Mice

Four mice from a litter of six, 15 days old, were each given 2·5 mg. of moist bacilli intraperitoneally. The remainder of the suspension was stored in the ice chest and the mice reinoculated with the same dose seven days later. The mice were killed at intervals of 9 to 34 weeks after the first inoculation, four smears were made from the small intestine, and one each from the liver, spleen, caecum, colon and rectum of each mouse. It will be seen from Table I that there were only a few bacilli in the liver and spleen of mice 1 and 6, killed nine and ten weeks, respectively, following inoculation, but in mouse 2 killed after 24, and mouse 5 killed after 34 weeks, the liver, spleen and almost the whole of the intestinal tract were heavily infected. Most of the smears from these sites revealed very large numbers of organisms. Cultures from the spleen of mouse 1 and the spleen and liver of mouse 2 were typical. When the latter were subcultivated they grew on Dunkin's medium but failed to grow on plain egg medium.

A 46-day-old culture from the spleen of mouse 1 was used to inoculate a further seven mice. Five of one litter (Nos. 23, 24, 25, 26 and 27) received 1 mg. of moist bacilli and two of another litter (Nos. 30 and 31) 0·5 mg. when 13 days old. Seven days later the five mice each received 0·4 mg. of bacilli and the two mice, *i.e.*, 30 and 31, were each given 0·75 mg. bacilli. Mouse 26 died within a few days of the second inoculation. Table I shows that in mice 24, 23 and 27, killed or dying 12, 15 and 19 weeks, respectively, after inoculation, the infection was developing normally, but that mice 25

and 31, killed 20 weeks after inoculation, had either recovered or infection had never become established. In mouse 30, killed 24 weeks after inoculation, only small numbers of bacilli were found in the intestinal tract but the liver and spleen were fairly heavily infected.

Direct Mouse Passage Experiments.—Portions of liver and spleen from mouse 1 were ground in a Griffith tube and suspended in saline. Four ten-day-old mice were each given intraperitoneally 0·1 ml. of liver suspension and a further four 0·1 ml. of the spleen suspension. Seven days later all eight mice received 0·1 ml. of liver suspension from mouse 6. Two of these mice died and were not examined, the remainder were killed or died 17 to 27 weeks after inoculation. Smears were made from the usual sites but no acid fast bacilli were found.

A saline suspension (1 : 4) was made with the liver of mouse 2 and seven 13-day-old mice (Nos. 32 to 38) from a litter of nine were given intraperitoneally 0·1 or 0·2 ml. of this suspension. Some of the mice were reinoculated seven days later. Mouse 33 died within a few days of the second inoculation. An examination of Table I shows that the infection developed in the other mice more rapidly than in those inoculated with the original culture.

Second Passage.—Fifteen mice, 14 days old, received intraperitoneally 0·05 gm. of antiformin treated liver suspension from mouse 32. Salmonella infection developed among them and all but one died within five weeks. In those dying towards the end of this period, however, infection of the liver, spleen and intestine appeared to be developing normally. The single mouse which remained alive was killed after eight months. Smears from the bronchial and mesenteric lymphatic glands revealed a few single acid-fast bacilli, and small intracellular groups. All the other smears were negative. A section of the liver, however, revealed lesions comparable in number and size with those in the liver of mouse 1. In two of these lesions there was an epithelioid cell containing a few degenerate acid-fast bacilli ; the surrounding cells were of the macrophage type. Sections of the intestinal tract did not reveal any acid-fast bacilli. It is not known whether the disease had at one time been more extensive in this mouse and then regressed. Twort (1914) has suggested that this occurs in rabbits and Hagan (1938) has described a cow that almost died from advanced Johne's disease but when killed five years later only had slight lesions in the caecum.

The pooled bronchial and mesenteric lymphatic glands weighed 0·14 gm. After treatment with 10 per cent. antiformin and washing, the deposited material was sown on four tubes of Dunkin's medium. A moderate number of colonies developed after six weeks' incubation. Viable bacilli were therefore recovered one year and four months after the original culture had been inoculated into mice.

The whole intestinal tract of mouse 2 was ground in a mortar and a saline suspension (1 : 4) was prepared. Approximately 0·02 ml. of this suspension was fed to each of nine ten-day-old mice. Three days later they received 0·04 ml. and in a further four days 0·08 ml.

TABLE I

Material used for intraperit. inoculation	*Age of Mice when first inoc. Days*	*First dose mg.*	*Interval between doses. Days*	*Second dose mg.*	*Time between first inocl. and P.M. Weeks*	*Mouse No.*	*Result of Smear Examinations from Various Tissues*								
							Liver	*Spleen*	*S.I.1*	*S.I.2*	*S.I.3*	*S.I.4*	*Caecum*	*Colon*	*Rectum*
40-day old culture from bovine mesenteric lymphatic gland	14	2·5	7	2·5	K 9	1	+	+	—	—	—	—	—	—	—
	„	„	„	„	K 10	6	+	+	—	—	—	—	—	—	—
	„	„	„	„	K 24	2	+++++	+++++	+++++	++	++++	+++++	+++	++	++
	„	„	„	„	D 34	5	++++	+++	++++	++++	+++++	++++	++	++++	+++++
One in four liver suspension from mouse 2	13	25*	—	—	D 5	35	+	+	—	—	—	+?	+	O	O
	„	50	7	50*	D 10	32	++++	+	—	—	—	—	—	—	—
	„	25	7	25	K 10	36	+++	++	O	O	+	O	+	O	+
	„	50	—	—	D 16	34	++++	+++	+++	++	+	+	+	+	+
	„	25	7	25	D 16	38	++++	++++	++++	+++++	+++++	+++	++	++	++
	„	25	7	25	D 17	37	++++	+++	++++	+++	++	++	++	+	+
46 day old culture from the spleen of mouse 1	13	1·0	7	0·4	K 12	24	++++	+++	++	++	++	+	—	+?	—
	„	„	„	„	D 15	23	++++	++++	O	O	++++	++++	+	+	O
	„	„	„	„	D 19	27	++	+++	+++	+++	++++	++++	++	++	+
	„	„	„	„	K 20	25	—	—	—	—	—	—	—	—	—
	„	0·5	„	0·75	K 20	31	—	—	—	—	—	—	—	—	—
	„	„	„	„	K 24	30	+++	+++	—	—	+	—	—	—	+

* = Weight of liver tissue injected.
K = Indicates that the mouse was killed.
D = „ „ „ died of salmonella infection.
*S.I.*1-*S.I.*4 = Smears of small intestine at intervals from stomach to caecum.

O = Tissue not examined.
—No organisms found in smear.
\+ = A few odd organisms or small groups.
++ = Organisms or groups found easily but not numerous.
+++ = „ „ fairly numerous in most parts of the smear.
++++ = „ „ very „ „ „ „
+++++ = Masses and myriads of organisms „ „ „

One mouse died ten weeks and five mice 15 weeks after receiving this material. They all died from salmonella infection. The usual smears were all negative except one from the upper part of the small intestine of the mouse dying ten weeks following infection in which two large masses of acid-fast bacilli were observed.

After examining smears from all the mice the impression has been gained that, following intraperitoneal inoculation, the bacilli first multiply in the liver and spleen and that the liver becomes the more heavily infected. Some time later multiplication of bacilli begins in the small intestine usually in the upper part, and spreads downwards towards the anus. Eventually bacilli are more numerous in parts of the intestine than they ever are in the liver. A more extensive examination was carried out on mice 37 and 38 than on those previously examined ; in both, fairly numerous bacilli were found in the mesenteric and bronchial lymphatic glands. In another mouse bacilli were observed in the bronchial lymphatic glands at a very early stage in the disease. In mice 37 and 38 a few bacilli were also found in the kidney, lung, pyloric portion of the stomach and heart blood, but in all probability they had only entered these organs just prior to death. Smears were not made from any other tissues.

Histological Findings in Mice

The amount of material examined histologically has had to be very limited and the following must, therefore, be accepted as a preliminary report.

Liver.—Following intraperitoneal inoculation the johne bacilli are taken up by cells of the reticulo-endothelial system throughout the liver. It appears that the bacilli then multiply intracellularly and that this stimulates the reticulo-endothelial cells containing them to divide or attract fresh cells which in turn become infected. It is quite probable that both these processes cause the growth of the lesion which gradually pushes aside the liver cells. In all sections individual Kupffer cells containing one bacillus or a small group of bacilli have been observed. Lesions occur in all parts of the liver lobules, even adjacent to the central lobular vein, but those in the portal tracts are the largest and when well developed consist of masses of epithelioid cells surrounding the blood vessels and bile duct. The cells are packed with masses of acid-fast bacilli, and in sections stained with haematoxylin and eosin it can be seen that those in the centre have reached the " symplasma stage " (M'Fadyean, 1918) and those at the periphery are transitional in type. Multinucleated epithelioid cells have also been observed. The circular aggregations of epithelioid cells within the liver parenchyma sometimes appear to be surrounded by a thin layer of true endothelial cells. No central necrosis has been observed and there is no connective tissue separating the lesions from apparently normal liver cells. In long standing infections many of the lesions observed have probably developed from nearby foci of infection where active multiplication of bacilli has occurred.

PLATE I. ×220. Liver of mouse 2 showing masses of bacilli in a portal tract and smaller groups in the surrounding liver tissue.

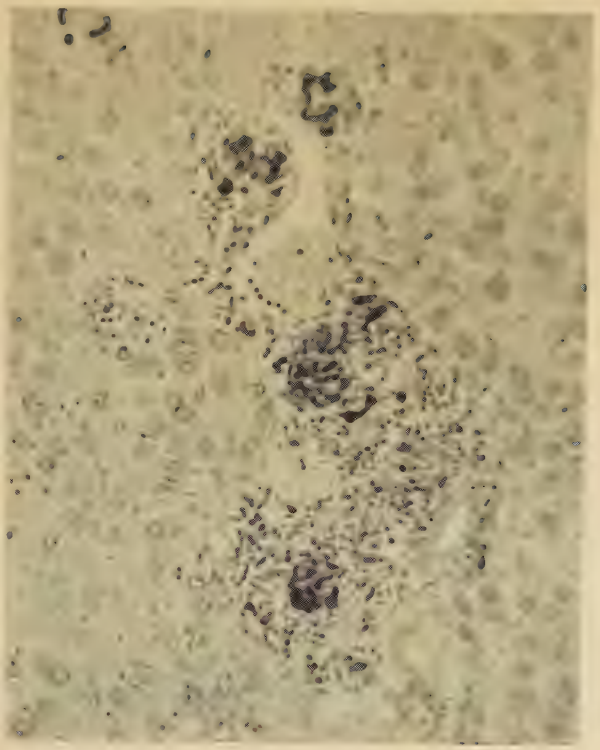

PLATE II. ×220. Liver of mouse 36. The same type of lesion as in mouse 2.

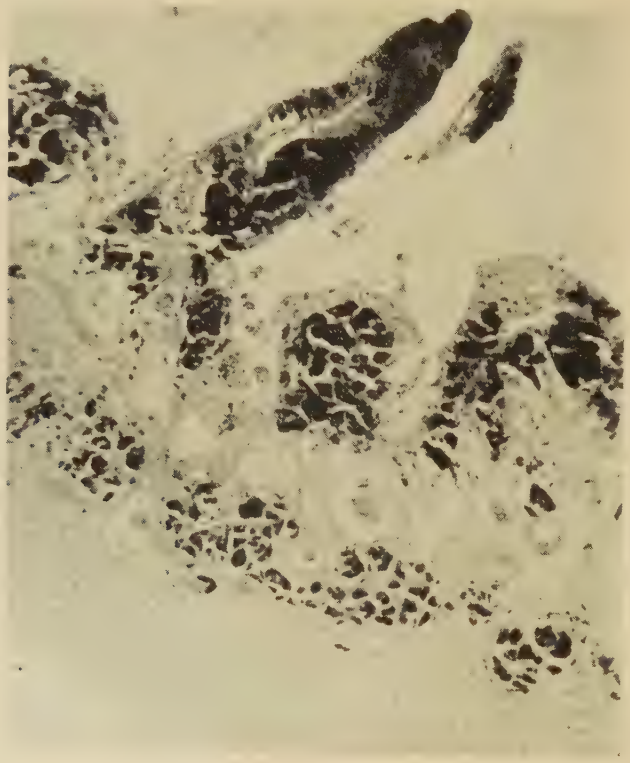

PLATE III. ×220. Small intestine of mouse 5 showing extensive invasion by bacilli. A few small groups can be seen in the muscular layer.

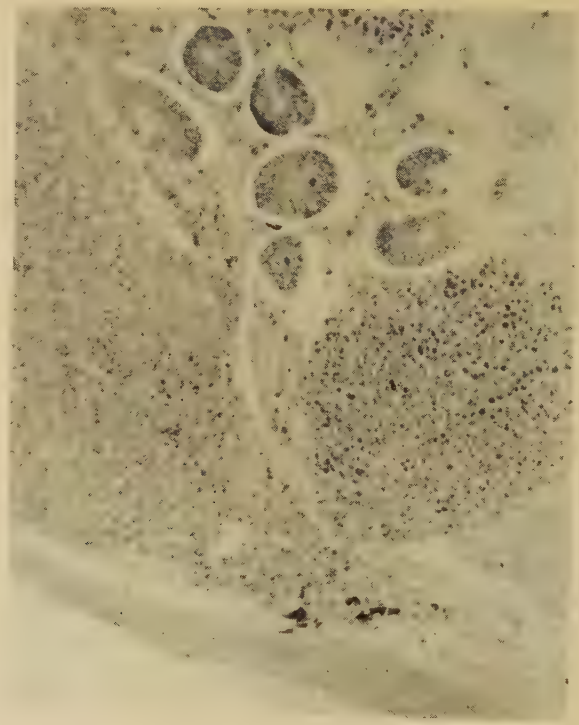

PLATE IV. ×200. Small intestine of mouse 36 showing four groups of bacilli just above the muscle layer and one group lying amongst the intestinal glands.

The difference between liver sections from mouse 1 and those from mouse 2 (Plate I) and mouse 5 was striking. The bacilli in mouse 2 and mouse 5 had undoubtedly multiplied and the size of the individual lesions had greatly increased. A comparison of liver sections from mice given a one-in-four liver suspension from mouse 2, with those receiving the original culture showed that the lesions in mouse 35 were more extensive than those in mouse 1, and in mouse 36 (Plate 2) the lesions had again increased in size, some of them being almost as large as those in mouse 2. It is surprising that despite the extent of the lesions no macroscopic changes have been observed in any of the livers except when death has been due to salmonella infection.

Intestinal Tract.—It appears that following intraperitoneal inoculation the bacilli enter the intestinal serosa. They then pass through the muscle layers and reach the submucosa where they multiply and eventually invade the mucosa. In advanced cases the villi are thickened and the mucosa and submucosa are packed with bacilli (Plate III). At the same time bacilli may be fairly numerous in the longitudinal and circular muscle layers and in the serosa. Small clumps of bacilli have been observed apparently within epithelial cells, but this may have been due to superimposition. The villi are often denuded of epithelium but this is probably a *post-mortem* change as mouse intestine is extremely friable. The usual masses of epithelioid cells in various stages of development have been observed. Bacilli and epithelioid cells also invade the lymphatic nodules of the intestine. The changes in the large intestine are similar to, but less extensive, than those in the small intestine. The number of bacilli in the muscle layer appears, however, to be greater. Masses of bacilli were observed amongst the muscle fibres in the colon of mouse 5 and in sections stained with haematoxylin and eosin corresponding groups of epithelioid cells were observed. In this mouse there was also a well defined layer of epithelioid cells covering the peritoneal surface of the rectum.

Hamsters (Cricetus auratus)

The same suspension as that used to inoculate the original mice was employed. Five hamsters, 23-day-old (Nos. 1, 2, 3, 5 and 6), with an average weight of 42 grammes, each received 2·5 mg. of moist bacilli intraperitoneally followed by the same dose seven days later. (On the basis of their respective weights this dose was approximately three times less than that received by the mice.)

Hamster 5, killed nine weeks following inoculation: Smears revealed a few acid-fast rods in the liver and fairly numerous bacilli in the spleen.

Hamster 6, killed ten weeks after inoculation: A few acid-fast rods were found in the spleen but liver smears were negative.

Hamster 1, killed 22 weeks after inoculation: Smears from liver and spleen were negative but well grown cultures were obtained from each of these organs. Smears from the ileocaecal region showed a

few acid-fast groups and a smear from a thickened Peyers patch in the lower jejunum showed numerous acid-fast bacilli. The whole intestinal mucosa was emulsified ; only a few odd groups of organisms were seen in a smear from this material.

Hamster 2, killed 31 weeks after inoculation : No acid-fast organisms were found in smears from any of the usual sites and cultures from the liver and spleen were negative.

Hamster 3, killed 32 weeks after inoculation : Smears from liver and spleen were negative but a smear from the lower part of the ileum showed a few epithelioid cells containing acid-fast bacilli. A smear from the enlarged mesenteric lymphatic glands showed numerous epithelioid cells crowded with acid-fast bacilli.

Direct Passage Experiments in Hamsters.—A thick emulsion was made from the spleen of hamster 5 and was inoculated in 0·1 c.c. quantities intraperitoneally into four ten- to 12-day-old hamsters (Nos. 10, 11, 12 and 14) with an average weight of 11 grammes and 0·2 c.c. into one three-month-old hamster (No. 15) weighing 95 grammes. A week later the hamsters were inoculated in the same way using the spleen of hamster 6. Two of the young hamsters died soon after inoculation and no *post-mortem* examination was made.

Hamster 10, killed twelve weeks after inoculation : Smears from the liver and spleen were negative but positive cultures were obtained from these organs. The whole of the intestinal tract was ground in a mortar and smears from this material were negative.

Hamster 11, killed 25 weeks after inoculation : In one smear from the upper part of the small intestine seven endothelioid cells were observed containing groups of acid-fast bacilli. A section of the lower part of the small intestine revealed a lymphatic nodule extensively invaded with single acid-fast bacilli. Epithelioid cells were observed in the corresponding section stained with haematoxylin and eosin. A section of the liver also showed some of the portal tracts containing epithelioid cells and a few acid-fast bacilli.

Hamster 15, killed 26 weeks after inoculation : Smears were entirely negative.

No young hamsters were available when the *post mortem* was performed on hamster 1, but four seven-day-old hamsters (Nos. 16, 17, 18 and 19) with an average weight of 102 grammes were anaesthetised with ether and each given 0·25 c.c. of the suspension of intestinal mucosa from hamster 1 by stomach tube.

Hamster 16, dead five weeks after receiving the suspension : All the smears were negative and an attempt to cultivate organism from the liver and spleen failed.

Hamster 17, killed 17 weeks after receiving the suspension : One small group of bacilli and one acid-fast bacillus were observed in a smear from the liver but all other smears were negative.

Rabbits

When the primary culture of *Myco. johnei* was five months old subcultures were made and incubated for 44 days. A saline suspension

was prepared and four rabbits, ten days old, were each inoculated intraperitoneally with 8·9 mg. of moist bacilli. Their average weight was 150 grammes. Seven days later three of the rabbits each received 17 mg. of moist bacilli from a similar culture. Smears from the liver and spleen and intestinal tract of one rabbit killed twelve weeks after inoculation were negative but positive cultures were obtained from the liver and spleen. The remaining three rabbits were killed 27 weeks after inoculation. There was a small caseous nodule in the mesentery of the rabbit which had received only one dose and eight caseous nodules which varied in size from half a pea to a mass 1·5 cm. in diameter were found in the mesentery of each of the two remaining rabbits. Smears from these nodules showed fairly numerous groups of acid-fast bacilli but smears from the organs and the intestinal tracts were negative.

Guinea-Pigs

The one in four liver suspension from mouse 2 which was used to infect mice was also used in an attempt to infect guinea-pigs. Two guinea-pigs, two days old, were given 0·4 ml. and two 0·2 ml. of the suspension intraperitoneally. Seven days later the two which had received 0·2 ml. were again given the same dose of the suspension and one guinea-pig received a second dose of 0·4 ml. All the guinea-pigs were killed approximately six months later. They had a few caseous nodules in the mesentery, which contained fairly numerous acid-fast bacilli but smears from the organs and intestines were negative.

One three-day-old guinea-pig was inoculated intraperitoneally with 0·45 gramme of antiformin-treated liver tissue from mouse 32. It was killed five months later. There were no *post-mortem* lesions nor were acid-fast bacilli seen in smears of the organs or intestine.

DISCUSSION

The following facts very strongly suggest that the culture employed was *Myco. johnei* and not any of the recognised types of *Myco. tuberculosis*. It grew characteristically on Dunkin's medium but failed to grow on plain egg medium. No caseous lesions were observed in any of the organs or lymphatic glands of inoculated animals though some were found in the mesentery of rabbits and guinea-pigs. Despite the large dose of organisms all the animals continued to thrive and a progressive disease was not produced in rabbits or guinea-pigs. The cellular reactions in affected animals were similar to those observed in naturally occurring Johne's disease. Further evidence that the transmitted disease was not tuberculosis is provided by the following results. A saline suspension was prepared from liver of mouse 35. Two guinea-pigs each received 0·1 gramme of actual liver tissue subcutaneously and one rabbit 0·2 gramme intraperitoneally. These animals were killed seven weeks after inoculation ; no macroscopic lesions were present and smears from

all the organs were negative. Similar results were obtained with liver tissue from mouse 38. It is of course unfortunate that salmonella infection developed among the mice, and it may have increased their susceptibility to Johne's disease but it should be noted that mouse 2 was killed before there was any evidence of disease in the stock.

There is now good evidence that even naturally susceptible animals develop a marked age resistance to Johne's disease. The mechanism of age immunity in Johne's disease is not understood but Sabin (1940) has gone far to elucidate the mechanisms responsible for the development of an age resistance to certain virus diseases. It is assumed but not proved, owing to the early death of inoculated adult animals, that infection was established in the mice and hamsters because very young animals were used. It may be that the strain of mice used, which were originally obtained from Dunkin, were particularly susceptible or that the culture was of abnormally high virulence. On the other hand more susceptible strains of mice and more virulent cultures may be found. To those familiar with tuberculosis a total dose of 5 mg. of moist bacilli may seem enormous but it is smaller than doses of *Myco. johnei* given by other workers who have obtained negative results. It is interesting that mice appear to be more susceptible to Johne's disease than guinea-pigs because it is well known that they are more resistant than guinea-pigs to tuberculosis.

The most striking features of the " disease " in mice are the lack of clinical symptoms or macroscopic *post-mortem* findings and the rapid multiplication of bacilli in the liver and spleen which occurs sooner than in the intestine. Twort (1914) has previously reported these findings but he only succeeded in infecting a small proportion of adult inoculated mice and performed no direct passage experiments. In naturally susceptible species bacilli do not apparently multiply in the liver or the spleen and it was believed that the intestinal tract and its associated lymphatic glands are the only parts of the body affected. Mathews (1930), however, claims to have observed specific liver lesions in affected cattle, and Dunkin (1938) states that the intestinal wall and its adjacent glands are probably the last place to be invaded and that primary infection probably occurs in some of the anterior skeletal lymphatic glands. The work of Boquet and Saenz (1930) supports this belief. It is quite likely that this is also the route of infection in many " enteric " diseases, and the classical experiments of Sanarelli (1925) have provided convincing evidence that in anthrax the intestinal tract is not primarily invaded but is secondarily infected *via* the blood stream and that *B. anthracis* cannot even multiply in the intestinal fluids.

It is proposed to continue this work and to use different strains of mice and of *Myco. johnei.*

SUMMARY

Four mice, 14 days old, were each given intraperitoneally 2·5 mg. moist bacilli from a culture of *Myco. johnei.* They were reinoculated

with the same dose seven days later. *Post-mortem* examinations were carried out 9 to 34 weeks after inoculation and showed that a progressive infection commencing in the liver and spleen and eventually involving almost the whole of the intestinal tract had developed. Seven mice, 13-day-old, received liver tissue from one of these infected mice. The bacilli multiplied in the second group of mice more rapidly than in those originally inoculated. The results of a second mouse passage were largely vitiated due to the early death of the inoculated mice.

Another group of 13-day-old mice was inoculated with a total dose of 1·4 or 1·25 mg. of culture from the spleen of an infected mouse. Three of these mice rapidly became heavily infected but two, killed later, were negative and a third was only lightly infected.

Slight infection was also produced in young hamsters, but on the basis of their weights the doses they received were approximately three times smaller than those given to mice. The results were less consistent but bacilli were carried through one direct animal passage.

The cellular reactions in infected mice and hamsters were similar to those in naturally completed Johne's disease.

In this study young rabbits and guinea-pigs appeared to be less susceptible to Johne's disease than mice or hamsters.

ADDENDUM

Since this article was completed for publication (February 8th, 1942) two abstracts have been seen. Borodenok, A. I. (*Vet. Bull.*, **12,** in press) found lesions of Johne's disease in the gall bladder, portal lymph nodes and spleen in two of 30 infected animals. Popp, P. (*Jber. Vet.-Med.*, **68,** 45) apparently infected several mice by the intravenous inoculation of pure cultures of *M. johnei*; intraperitoneal inoculation did not succeed unless the organisms were suspended in liquid paraffin. Attempts to infect two guinea-pigs failed.

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NOTES ON TETRATHIONATE BROTH AND MacCONKEY'S AGAR MEDIA IN THE ISOLATION OF *SALMONELLA ENTERITIDIS* VAR. *DUBLIN* FROM BOVINE FAECES AND MILK

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DURING the summer of 1940 an outbreak of illness in calves caused by *Salmonella enteritidis* var. *dublin* was investigated in this laboratory (Craig et al., 1941). The media used for the isolation of the causal organisms were : tetrathionate broth, MacConkey's agar, and, in some cases, Wilson and Blair's medium. The same methods were used in examining the milk and faeces of the milking herd on the farm in an attempt to discover the presence of a carrier of the causal organism. None was found.

It was thought that it would be useful to make some quantitative tests of the methods used in the isolation of the organisms in order to obtain a rough estimate of how heavy an infection requires to be present before it can be detected. The experiments consisted in adding measured quantities of successive dilutions of broth cultures of *S. enteritidis* v. *dublin* to fresh milk and to suspensions of faeces in normal saline, and testing these artificially infected materials by (*a*) plating out directly on MacConkey's agar medium, and (*b*) inoculating into tubes of tetrathionate broth and plating from these after incubation. Plate counts were made from the culture dilutions at the same time, and the approximate number of organisms per c.c. of milk or faeces suspension, computed from these counts.

Wilson and Blair's bismuth medium (Tabet, 1938 ; Wilson and Blair, 1927 and 1931) was also used in these experiments, but the results are not given here as they were conflicting. The conclusion was reached, however, that this medium has no advantage over MacConkey's medium in the isolation of *S. enteritidis* v. *dublin* and, in fact, is often inferior to MacConkey's medium. Wilson and Blair (1927) state that *S. enteritidis* was completely inhibited by their medium as it was then made up. The same authors (1931) later state that *S. enteritidis* grew well on their medium but required four days' incubation before typical colonies appeared. One batch of our medium, made up according to Tabet's modification, gave extremely poor results as compared with MacConkey's medium. Two subsequent batches gave good results, but two batches borrowed from another laboratory (made up according to the same method) gave very poor results.

The Media Used in the Tests

MacConkey's agar medium was made up in the usual way. In the earlier tests it was not standardised but after the trouble noted

below when a different brand of peptone came into use, it was accurately standardised to *p*H 7·2 to 7·4.

In the earlier experiments the tetrathionate broth was made up according to the method described in Bigger's "Handbook of Bacteriology" (5th edition), but later a somewhat simpler method was used, giving exactly similar results. In this latter method 2·5 gm. of chalk (pure precipitated) are added to 90 c.c. sterile broth and steamed for half an hour. When cold, 10 c.c. of a 60 per cent. solution of pure crystalline sodium thiosulphate, previously steamed for half an hour and cooled, and 2 c.c. of an iodine solution, consisting of 6 gm. iodine, 5 gm. potassium iodide, and water up to 20 c.c., are added. The medium is tubed in 5 c.c. lots by pipetting, the flask being kept swirling. It must be used within three weeks of preparation (Muller, 1923; Jones, 1936).

This medium is simple to prepare and is in routine use in many medical laboratories for the isolation of the enteric and food poisoning group of organisms.

METHOD OF CARRYING OUT TESTS

A. Faeces

Fresh normal bovine faeces, in 2 gm. amounts, are weighed and put into sterile short wide tubes. To each tube 7 c.c. sterile saline is added. Then 15 progressive five-fold dilutions of a four- to five-hour broth culture of *S. dublin* are made with peptone water as diluent, and 1 c.c. of each dilution is added to a tube of faeces. These tubes are then stoppered and each shaken violently for about half a minute. These infected faecal suspensions are now plated out on MacConkey's agar medium, a large (3-4 mm. diam.) loopful being spread on each plate.

Then 1 c.c. amounts of the suspensions are added to tubes of tetrathionate broth.

At this stage 0·5 c.c. quantities of the peptone water dilutions of the broth culture are put into plates and mixed with 12 c.c. melted nutrient agar for counts, three plates being made from each dilution. (After a few preliminary tests, counts from dilutions at the beginning of the series were omitted.) The counts are read after 24 hours' incubation. The remainder (2·5 c.c.) of the peptone water dilutions are incubated along with the other tests. (See notes on counts later.) The tetrathionate broths are incubated for 18 hours and then plated out on MacConkey's agar medium. The MacConkey plates, direct and from tetrathionate broth, are read after 24 hours' incubation.

B. Milk

Much the same method was used, but twofold dilutions of the culture were added to 19 c.c. fresh untreated milk in bottles. For the counts in the first test, only one plate was used for each dilution, and in the second test, two plates. (The milk tests were done at an earlier period than the faeces tests, before the technique had been standardised.)

Results

The results of the faeces tests are given as " Organisms per c.c. faeces emulsion," rather than " per gm. solid faeces," as the latter would be misleading. In routine work a thicker suspension of faeces is used, about 1 gm. to 2 c.c. saline, for spreading directly on plates, and about 0·5 gm. solid faeces is added to a tube of tetrathionate broth. A comparatively thin suspension of faeces was used in these tests in order to ensure even distribution of the organisms through the mixture. In the tests, as in routine use of the media, more material was put into the tetrathionate broth than could be spread on a plate, but in routine work the amount going into the broth is even larger compared with the amount that can be spread over a plate, or even several plates.

The results of faeces Test 1 are given in full (Table A). The end point only of subsequent tests are tabulated (Tables B and C). The number of organisms per c.c. of faeces suspension, given as the end points, are, of course, only intended to be taken as estimates.

TABLE A

Plate Number	*Results of Faeces Test* 1		*Approximate Number of Organisms per c.c. Faeces Suspension as computed from Plate Counts*
	" Dublin " Colonies present on :		
	Direct Plates	*Plates Spread from Tetrathionate Broth*	
5	+	+	40,625
6	+	+	8,125
7	+	+	1,625
8	—	+	325
9	—	+	65
10	—	+	13
11	—	+	2·6
12	—	—	—

With the direct plates, the early plates of the series show a heavy growth of salmonella colonies, with very few lactose fermenters. As the series progresses, the salmonella colonies tail off, and the end point usually shows two or three colonies amidst a heavy growth of lactose fermenters. The plates from the tetrathionate broth show a heavy, pure or almost pure growth of salmonella colonies right up to the end point, where they cease abruptly.

The results of Tests 2 and 3 (Table B) show the " trouble " with the MacConkey's medium referred to before. The direct plates were satisfactory, but the plates spread from the tetrathionate broth showed, in Test 2 no growth at all, and in Test 3 very scanty growth of salmonella colonies. Wilson and Blair plates, spread from the same tubes of tetrathionate broth, showed a heavy growth of dublin up to Plate II of the series in both cases, representing in

Test 2, 4 organisms per c.c. faeces suspension, and in Test 3, 1·44 organisms per c.c. Tests of the new peptone which had come into use showed it to be very acid and when this was corrected the MacConkey plates gave good results, as seen in Tests 4, 5, 6 and 7. Possibly the reason why the direct plates were fairly satisfactory, even with the acid MacConkey, was that on them the organisms were mixed with a large amount of faecal matter, which may either correct the medium somewhat or protect the organisms from the inhibitory effects of the acidity of the medium. It will be seen, also, that the direct plates in Tests 4, 5, 6 and 7 show better results than those in Tests 1, 2 and 3.

The colour of the medium when neutral red is added does not seem to be a sufficiently delicate test of its *p*H. Jones (1936) also states that MacConkey's medium should be standardised more carefully than is usually done.

TABLE B

RESULTS OF FAECES TEST 2 TO 7

Test Number	*Medium*	*Final Plate Showing "Dublin" Colonies*	*Organisms per c.c. Faeces Suspension*
2	Direct plates	No. 7. 2-3 colonies	2,500
	Ex. T. broth	All negative	
3	Ditto	No. 7. 2-3 colonies	900
		No. 7. Very scanty growth	900
4	Ditto	No. 9. 2-3 colonies	50
		No. 11. Heavy growth	2
5	Ditto	No. 8. 2-3 colonies	255
		No. 12. Heavy growth	0·41
6	Ditto	No. 9. 1 colony	30
		No. 10. Heavy growth	6
7	Ditto	No. 10. 1 colony	15
		No. 10. Heavy growth	15

N.B.—"Ex. T. broth" = plates spread from tetrathionate broth.

Notes on the Counts

In each test the group of three plates showing the most convenient number of colonies for counting was chosen, *i.e.*, between 50 and 300. The method of working out the number of organisms per c.c. faeces emulsion is shown in full below for faeces Test 1. The corresponding figures for all the tests are given in Table D.

Test 1

The three plates made from dilution 10 of the series contained 65, 62 and 67 colonies respectively. Average 64·666 = 65. As 0·5 c.c. quantities of peptone water dilution were used for the counts, dilution 10 contains 130 organisms per c.c.

1 c.c. of culture dilution was added to a tube containing 2 gm. of faeces + 7 c.c. saline. Therefore the faeces emulsion contains 13 organisms per c.c.

TABLE C

RESULTS OF MILK TESTS

Test Number	*Medium*	*Final Plate Showing "Dublin" Colonies*	*Organisms per c.c. Faeces Suspension*
1	Direct plates	No. 13	448
	Ex. T. broth	No. 18	14
2	Ditto	No. 7	2,064
		No. 16	4

The incubation of the remains of the peptone water dilutions was carried out in order to serve as a rough check on the counts. One cannot say exactly how many organisms might be present in a tube of peptone water for growth to take place, but it is presumed that this would be a "small number," perhaps between one and five. The following figures and table are therefore appended in order to give an estimate of the error involved in these counts.

In Test 1, according to the count (Table D), peptone water dilution 10 should contain 130 organisms/c.c., therefore peptone water dilution 13 should contain 1·04 organisms/c.c. Dilution 13 was the last tube in the series to show growth after incubation.

COUNTS. TABLE D

Test No.	*Plate No.*	*Counts*			*Average*	*Final Tube Peptone Water should Contain*	*Tube No.*
1	10	65	62	67	65	1·04 organisms/c.c	13
2	10	97	94	105	99	1·58 „ „	13
3	9	176	189	177	180	2·88 „ „	12
4	9	251	218	288	252	0·81 „ „	13
5	9	270	255	244	256	4·10 „ „	12
6	9	146	152	159	152	2·43 „ „	12
7	10	74	82	75	77	0·25 „ „	14

Notes on the Results

In Tests 1 to 5, and in the two milk tests, results were taken from examination of the plates only. In view of the fact that a known organism was added to healthy faeces or milk, and that the end few plates of the series always acted as negative controls, further verification of typical colonies was not considered necessary. Any doubtful colonies were subcultured on fresh MacConkey's plates. In Tests 6 and 7, however, several colonies from each of the later plates of the series were verified by the agglutination test with "Dublin" serum. In no case did the agglutination test disagree

with the diagnosis made from examination of the plates. All colonies picked as "Dublin" agglutinated the serum to titres (1:40,000).

The direct MacConkey's plates sometimes showed doubtful colonies, particularly near the end point, where a very few salmonella colonies were situated amidst a heavy growth of lactose fermenters, but when subcultured to fresh MacConkey's plates, the pure cultures were easily distinguishable as salmonellas or otherwise. The doubtful colonies were usually late lactose fermenters.

As the growth on the plates spread from the tetrathionate broth was always heavy and practically pure, right up to the end point, no doubtful colonies were encountered on these plates. The only colonies seen on plates made from tetrathionate, other than salmonella, were tiny pink ones consisting of cocci.

SUMMARY

1. Tetrathionate broth will give a pure heavy growth of *Salmonella enteritidis* var. *dublin* from infected bovine faeces and milk when the infecting organism is present in very small numbers. The smallest number of organisms per c.c. artificially infected faeces suspension, to be detected by the use of tetrathionate broth, varied from approximately 15 to 1. (Error involved shown in Table D.)

2. MacConkey's plates spread directly from infected faeces and milk usually require an infection five or ten times heavier to be present before they will detect the organism. In these cases only a few salmonella colonies may be present and these may well be overgrown by the normal flora of the faeces.

The smallest number of organisms per c.c. faeces suspension to be detected by direct plating on MacConkey's medium varied from approximately 255 to 15.

3. A much larger quantity of faeces or milk may be inoculated into a tube of tetrathionate broth than can be spread on a plate, thereby increasing the chances of recovering the organisms.

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THE CHICK RED CELL AGGLUTINATION TEST WITH THE VIRUSES OF NEWCASTLE DISEASE AND FOWL PLAGUE

By

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Since Hirst (1941 and 1942) described the agglutination of chick red cells by the influenza viruses, it has become of interest to find out whether any other viruses were capable of causing a similar effect. Burnet (1942) has found that the virus of Newcastle disease of fowls causes agglutination of the chick red cells in a manner very similar to that shown with the influenza viruses, and suggested (personal communication) that, as fowl plague virus was not available to him, tests should be made with it here. In this paper the results of the investigation are reported, and it will be shown that by this method a very quick differential diagnostic test can be made.

Materials

Both viruses were kindly supplied by Mr. N. Dobson through Professor T. Dalling of the Veterinary Laboratory of the Ministry of Agriculture, Weybridge. The Newcastle disease virus was in the form of a portion of infected fowl liver preserved in 50 per cent. glycerine; the fowl plague virus was received in the form of dried fowl liver. Sera from fowls which had been immunised against each disease and had then withstood a test dose of living homologous virus were also very kindly supplied by Mr. Dobson.

Methods

Passage in Eggs.—In this investigation the virus was propagated only in the developing hen's egg. Eggs were inoculated allantoically with 0·1 c.c of infective material after 11 or 12 days preliminary incubation. After 24 hours incubation at 36° C. the eggs were opened and the allantoic fluid drawn off, particular care being taken to avoid haemorrhage. Both viruses, but particularly fowl plague, are rapidly fatal to the embryo, and passage was usually made with a 1:100 or 1:1,000 dilution of allantoic fluid in broth in order to keep the embryo alive for 24 hours or a little longer.

Red Cell Agglutination.—This test is based on the observation made by Hirst (1941) that allantoic fluid from eggs infected with the influenza virus causes the agglutination of the red cells of the chick, and of adult fowl red cells. He further observed that the addition of immune serum to the infected allantoic fluid inhibited its ability to agglutinate the cells. He worked out a quantitative serum-inhibition test (1942), and his technique for setting up the test has been used throughout this work. For reading the results of the test Hirst estimated the amount of red cell sedimentation from the reduction of opacity of the supernatant fluid, as he found

this to be more easily determined than the degree of directly visible agglutination. When the virus of fowl plague is used, this method is satisfactory, but with Newcastle disease virus this method of reading the test is of little use. The reason for this is that, although there is definite agglutination as judged by precipitation of cells on the floor of the tubes, this affects only a small proportion of the cells, so that the density of the suspension is not greatly reduced, and even the most concentrated virus suspension seldom shows reduction of opacity beyond Hirst's one plus (+) reading. Measurement of the amount of agglutination had, therefore, to be made and was assessed as follows: ++++ = Continuous sheet of agglutinated cells, often ragged at the edges and covering almost the whole floor of the tube. +++ = Similar in type, but less heavy. ++ = Moderate precipitation, usually widely spread over bottom of tube, the precipitate not continuous as a sheet, but broken into discrete particles. + = Similar in type, but less in amount than ++, sometimes showing as a rather ragged outline to the central round spot of normally sedimenting cells. ± = Only a trace of agglutination. The necessity of reading the Newcastle disease tests by precipitation is illustrated in Table I and, for the sake of conformity, those with fowl plague were also read by this method.

Serum Inhibition Tests.—Although there has never been any evidence to suggest that any serological relationship exists between the viruses of fowl plague and Newcastle disease, it was thought of interest to do cross tests with the respective immune sera and viruses. Table II shows the results of these tests, and it is obvious that the viruses are serologically unrelated.

DISCUSSION

The red cell agglutination test is to some extent still in its trial stages, its particular interest being that a method is now available for making certain tests *in vitro* which previously have had to be made in animals. The test as applied here to the viruses of Newcastle disease and fowl plague makes it obvious that modifications in Hirst's technique will be necessary, at any rate for some viruses. Why such differences occur is not as yet explained; the problem calls for the early attention of the physical chemists.

While there has never been any great difficulty in determining whether an outbreak of disease amongst fowls has been caused by Newcastle disease virus or by fowl plague virus, conditions might easily arise when it would be extremely useful to have at hand a quick method for laboratory diagnosis. This becomes available with the use of the red cell agglutination test. Any laboratory possessing a stock of immune sera against the respective viruses and some fertile hens' eggs should be able to give a diagnostic report within 24 hours, or at the very latest 48 hours, of receiving specimens from diseased fowls. Both diseases are highly infectious for fowls as well as having a very high mortality rate for the infected birds, and so

TABLE I

RED CELL AGGLUTINATION TITRATIONS READ BY "SEDIMENTATION" (HIRST) AND AGGLUTINATION

Antigen	*Antigen Dilutions*							*Control* 1 % *r.b.c.*	*Method of Reading Test*	*Titre*
	1/10	1/20	1/40	1/80	1/160	1/320	1/640			
Fowl plague ...	++++	++++	++++	+++	++	+	O	O	"Sedimentation" (Hirst)	1/160
" ...	++++	++++	++++	+++	+++	++	±	O	Agglutination	1/320
Newcastle disease ...	+	+	+	+	±	O	O	O	"Sedimentation" (Hirst)	<1/10
" " ...	++++	+++	+++	+++	++	±	O	O	Agglutination	1/160

The ++ reading is taken as the endpoint for both methods.

TABLE II

SERUM INHIBITION TESTS (RESULTS READ BY AGGLUTINATION)

Immune Serum	*Antigen*	*Serum Dilutions (Final)*									*Serum control*	*Antigen Control*	*Red Cell Control.*	*Serum Titre*
		1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096				
Newcastle disease	N.D.	—	—	—	O	O	O	±	++	+++	O	++++	O	1/2048
	F.P.	++++	++++	++++	++++	++++	++++					++++		<1/16
Fowl plague	N.D.	+	++	++	+++	+++	++++				O			1/32
	F.P.	—	—	—	O	O	O	O	++	+++				1/2048

far no small laboratory animal has been found which is suitable for experimental work. Rigid isolation precautions have always been taken to prevent the spread of these diseases in the laboratory, and the red cell agglutination test provides a useful method of handling these two viruses without the necessity of using fowls or chickens, except in the egg at an early stage of development.

SUMMARY

1. Hirst's red cell agglutination test has been shown to be applicable to the viruses of Newcastle disease of fowls and of fowl plague.

2. Serum-inhibition tests show that there is no serological relationship between the viruses.

3. The test provides a useful method for the rapid laboratory diagnosis of either disease.

ACKNOWLEDGMENTS

I would like to thank Sir Henry Dale, Director of this Institute, and Professor T. Dalling, of the Veterinary Laboratory, Ministry of Agriculture, Weybridge, for their permission to work with these viruses, and for the interest they have shown in the work. I am especially grateful to Mr. N. Dobson, also of the Veterinary Laboratory, for providing both the viruses and the immune sera used.

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YEAST AS A PROTEIN SUPPLEMENT FOR PIGS AND ITS RELATION TO THE APPEARANCE OF RICKETS

By

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INTRODUCTION

In the past, yeast has been regarded in this country as an accessory foodstuff, mainly a source of vitamins, and as such its use was restricted to small quantities. In time of war yeast acquires additional importance since besides its value as a source of vitamins, it can be used as a protein supplement for man and animal. It can be manufactured on a large scale, and so provide essential protein, should other sources partly fail. The amount of yeast which has to be consumed by an animal to cover its protein requirement is naturally much higher than when it is fed only as accessory nutrient. In Germany yeast has been used for some time as a protein supplement and vast quantities of it have been and are being produced for human use and for animals. The German experience, although valuable, is not necessarily applicable to British conditions, mainly because the wood-sugar (xylose) yeast so extensively used in Germany cannot be produced here on a large scale owing to the shortage of wood from which xylose yeast is manufactured. In this country brewers' yeast and fodder yeast are, or could be made, available in large quantities, and it was the object of the present experiments to provide more information about their nutritive value. A detailed review of the literature on dried yeast as fodder for livestock has been prepared by one of us (R. B.) and has been published separately (Braude, 1942).

In an experiment already reported, Braude and Foot (1942) found brewers' yeast an excellent protein supplement for fattening pigs when fed in conjunction with bulky feeding stuffs in the so-called Lehmann method of pig fattening. As a further step we have tried to ascertain whether yeast can wholly replace the protein supplement of peace-time pig rations, and whether any difference could be observed between three different yeast varieties: ordinary brewers' yeast, debittered brewers' yeast and fodder yeast. The debittered brewers' yeast was tried because of the prevalent opinion that removal of the bitter taste renders yeast more palatable, an advantage especially valuable when the feeding of large quantities is contemplated.

During the progress of the present experiment some difficulties resulting from feeding large quantities of yeast were observed, and we had to extend the scope of our work to include an investigation

into the causes and possible correction of the troubles experienced. This paper covers the main part of our investigation ; further experiments are still in progress, and will be reported later.

EXPERIMENT 1

The method of individual feeding adopted for this experiment was designed to compare the nutritive value of the three yeast varieties mentioned above as well as to compare them with meat meal which is in common use as a protein supplement in meal mixtures for fattening pigs.

Large White, home-bred weaners, 11-12 weeks old, were used. They were reared according to our usual routine, which includes folding of sows and litters in small units and additional feeding to the litter from the fourth week of a sucklers' meal which contains cod-liver oil. After weaning at eight weeks the animals were kept indoors.

Four diets were used and four pigs, one allocated to each diet, formed a single experimental block. All four pigs constituting a block were littermates selected to be as alike as possible at the beginning of the experiment. Altogether eight blocks were used, *i.e.*, each treatment was replicated eight times. Each block was started as pigs became available ; thus the members of each block started at the same time, but the last block started several weeks after the first. The initial live weight of the pigs varied slightly, but the average initial weight of the eight pigs on each treatment was about 58 lb. The distribution of the pigs within a block was by lot. Each block occupied a large pen which contained small pens for individual feeding. The pigs were placed in the individual feeding pens twice daily for the short time taken by the meal, after which they were allowed to run together in the large pen.

The experiment commenced in June, 1940, and was completed in January, 1941. The experimental period for each block was 16 weeks (112 days) except for a few pigs, when it was extended for special studies.

The normal control diet consisted of 46 parts of weatings, 20 parts each of barley meal and flaked maize, two parts of dried grass, 1½ parts of ground limestone and ½ part of salt, all parts by weight. In the three experimental diets the meat meal was replaced by eight parts by weight of yeast—ordinary brewer's yeast,* debittered brewer's yeast,* or fodder yeast†—and the weatings was increased from 46 to 48 parts in order to make up the diet to 100 parts.

All the meal mixtures were mixed by hand in the piggery. The chemical analyses of each of the food components are given in Table I. It will be noted that care was taken to provide both additional minerals (limestone and salt) and a source of carotene (lucerne meal). The meal was weighed out and fed twice daily in the form of a wet mash, 3 lb. of water being used for each pound of meal. The quantity of meal fed daily was based on the live weight of each pig each week and on the scale for fattening pigs recommended by the Pig Experiments Co-ordination Committee (quoted by Braude and Foot, 1942).

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* Purchased from Messrs. Arthur Guinness & Son, Ltd.

† Kindly prepared by Dr. A. C. Thaysen of the Department of Scientific and Industrial Research.

Results. Table II gives details of the growth of the experimental animals. Except in two cases (cf. footnote to Table II), all the pigs on the experiment made very satisfactory progress until two to three weeks before reaching the required bacon weight (13-14 weeks on the experimental diets), when some of the animals receiving yeast began to behave abnormally. Symptoms developed in 11 of the 24 pigs after a period on the experimental diet varying from 91 to 102 days. The first symptom was failure to consume the whole of the food supplied, and this was followed in six of the pigs by stiffness and lameness. These pigs walked on their toes with the knees bent and knuckling over; the hind limbs were used stiffly and the gait was straddling in type. The back was arched and the animals were very reluctant to rise, although the stiffness improved slightly when a pig was forced to take exercise. Finally, the most severely affected pigs went down on their knees and had to be held at the trough so that they could eat. Urination and defaecation were suppressed, but were readily induced by lifting the animals on to their feet. No similar symptoms were observed in any of the pigs receiving the control diet. Table III summarises the information concerning the affected pigs. It will be seen that the trouble always started with refusal of food, and that the next stage characterised by leg symptoms appeared in only six pigs.

No difference due to the variety of yeast was observed, the pigs being similarly affected on each of the three yeast diets. It will also be noticed that the adverse symptoms developed in pigs belonging to five different litters, while in the remaining three no abnormal symptoms appeared (Blocks 4, 5 and 6). Table II shows the average amount of food consumed by the pigs on each treatment as well as the food consumption per pound live weight gain. As the symptoms developed very late in the experimental period the effect on food consumption was small and there is only a negligible difference between the treatments; in general the food utilisation by all the pigs on the experiment may be regarded as satisfactory.

The food was eaten eagerly by all the animals until the appearance of abnormal symptoms, the first of which was reduced appetite. Table IV, showing the number of food refusals by pigs on each treatment, as well as the approximate amount of food refused, indicates clearly that the higher refusals were connected with the presence of yeast in the diet. The amount of food refused by the control pigs can be regarded as normal.

Search for an Explanation of the Abnormal Symptoms. A number of investigations were undertaken in an endeavour to trace the cause of the disturbances, which appeared to us to be almost certainly associated with the feeding of yeast. The first step was a more detailed enquiry into the composition of the diets and especially of the yeasts. An examination of the latter showed that no living yeast cells were present in the debittered brewers'

yeast and in fodder yeast, while only a very small number of living cells were found in the ordinary brewers' yeast. The presence of such minute numbers was considered of no importance.

A suggestion that the trouble was due to metal poisoning was proved to be unfounded by analysis of the three varieties of yeast for heavy metals, kindly carried out for us by the laboratory of Messrs. Arthur Guinness & Son, Ltd. The figures in Table V show the absence of toxic concentrations of heavy metals. Fluorine poisoning was ruled out by an analysis kindly carried out by Dr. H. H. Green, Veterinary Laboratory, Weybridge, on samples of urine collected from two badly affected pigs (Nos. 1527 and 1536). The urine from pig 1527 contained 0·3 mg./100 ml., while that of pig 1536 contained only 0·1 mg./100 ml. of fluorine. Commenting on these results Dr. Green considered the content quite normal.

The next problem was to ascertain whether the mineral balance in our diet was normal. Unfortunately the Ca and P content of the diets was not determined beforehand and the actual sample of meat meal used in the control diet was no longer available when it was decided to investigate the adequacy of the mineral balance. It may be assumed from its ash content (4·5 per cent., Table I) that the meat meal contained some 0·3 per cent. Ca and about 0·3 per cent. P. The yeasts and the basal mixture of all components without the protein concentrate were subsequently analysed and from these data the Ca and P contents of the different diets have been calculated and the probable values are given in Table VI. These figures show that the control diet was lower in P and had therefore a higher Ca : P ratio. Nevertheless, at this stage of the experiment and in view of our own experience and of that quoted in the literature, we considered that the amount of Ca and P as well as their ratio could be regarded as satisfactory in all the diets. This point was, however, taken into consideration when planning the second experiment.

The possibility that the abnormal symptoms were due to rickets was nevertheless considered and two pigs, both showing symptoms (pig 1447 in block 1 and pig 1467 in block 2) were given 28,000 I.U. of vitamin D as a solution of calciferol on three consecutive days. Pig 1447 did not improve and was slaughtered a fortnight later. Pig 1467 was, unfortunately, given manganese at the same time (cf. p. 165). Its condition remained unchanged for about three weeks when the pig started gaining weight and the leg condition improved, but did not clear up completely.

At slaughter a blood sample of the pigs of block 2 receiving the yeast diets was collected and analysed for Ca and P. Calcium was determined by the Clark and Collip (1925) modification of the Kramer and Tisdall method, and inorganic phosphorus by the Fiske and Subbarow (1925) method. The results are given in Table VII. In the same table values are included for pigs 1521, 1527 and 1532 obtained at a later stage in the post experimental period, and also

values for pigs on normal rations obtained simultaneously. These are discussed on p. 179. Both the Ca and P figures for pigs 1467, 1468 and 1470 were lower than those for the pigs on the normal diet, but no connection with the appearance of symptoms is apparent. The higher Ca in the blood of pig 1467 may have been due to the action of calciferol which the pig received a few weeks before it was slaughtered.

The vitamin B_1 and manganese contents of the experimental diets were then studied, since Perla (1939) has shown that in rats the metabolism of these two substances is interrelated and the consumption of large quantities of vitamin B_1 may cause untoward symptoms unless the intake of manganese is increased. Yeast is a rich source of vitamin B_1 and it occurred to us that the high vitamin B_1 content of the diet might be responsible for the adverse symptoms. All three yeast varieties were examined for vitamin B_1 and from the figures obtained the amount of vitamin B_1 contributed to the daily rations by the yeast was calculated for pigs of 100 and 200 lb. live weight (Table VIII), thus giving an estimate of the amount actually consumed by the experimental pigs at the different stages of the experiment. The diets were also analysed for manganese and the results are given in the same table.

It was decided to investigate the effect of addition of manganese to the yeast diets of every alternate block of pigs in our experiment. At the time of introduction of manganese the blocks were in different stages of the experimental period, and it so happened that in block 2 the animals were actually showing symptoms when extra manganese was given. After a fortnight, when the experimental period of this block came to an end, we decided to continue the yeast pigs for another four weeks to follow any curative effects of manganese. With blocks 4, 6 and 8 manganese was given in the hope of preventing the appearance of the adverse symptoms. It was given as manganese sulphate, added to the diet at a rate of 3 g. per day for a period which for blocks 2, 4, 6 and 8 respectively lasted 42 days (14 and 28 days' extension), 48, 89 and 89 days.

The result of the manganese treatment was negative. Pig 1467 given manganese while showing symptoms improved slightly, but recovery was not complete and may have been due to the fact that the pig had previously received calciferol. Its block-mate, pig 1468, which received only manganese did not show any response. Addition of manganese to diets of pigs 1521 and 1527 in block 8 over a period of 89 days did not prevent the occurrence of the abnormal symptoms.

Although the experimental rations were thought to supply sufficient vitamin A it was decided to estimate the vitamin A content of the livers of some of the animals in order to exclude the possibility of vitamin A deficiency. The findings are given in Table IX. The figures leave no doubt that the amount of vitamin A provided in the diet was satisfactory, and there seems to be no correlation

between the occurrence of symptoms and the vitamin A content of the livers.

Post Experimental Period. It will be seen from Table III that only six pigs showed leg symptoms and it seemed probable that some of the pigs may not have had time to show them owing to the shortness of the experiment. It was therefore decided to keep four pigs from the two youngest blocks receiving yeast for a further period to see whether they would eventually show symptoms and also to observe the progress of the disease in affected animals. For this purpose pigs 1532 (block 7) and 1527 (block 8), both receiving the diet with ordinary yeast, were chosen, the first being apparently normal, while the latter was showing symptoms (cf. Table III). Pigs 1536 (block 7) and 1521 (block 8), receiving debittered yeast, were also selected ; in this case the former showed symptoms (cf. Table III) while the latter was not affected.

During the first three weeks of this additional period no change in condition of the pigs was noticed. It was then decided to increase the proportion of yeast in the diet from 8 to 20 per cent. The composition of the new diet is given in Table X. The introduction of the modified diet caused no immediate deterioration in the health of the animals and, in fact, the lameness became less evident. Gradually, however, the appetite of the pigs became poorer, and, finally, after 35 days on the 20 per cent. yeast diet all the pigs refused food and rapidly lost weight and condition. At this stage some ordinary fattening meal not containing yeast was offered to two of the pigs. A full trough was rapidly eaten, though at the same time the 20 per cent. yeast diet was left untouched.

For the next fortnight the pigs were given a diet of one-half normal fattening mixture and one-half 20 per cent. yeast diet. This meal was consumed readily and three of the animals quickly made up the loss in weight incurred during the period of refusal of food. Pig 1536, however, was in such poor condition that it was decided to kill it and carry out a detailed post-mortem examination. The remaining three pigs were slaughtered a week later. The Ca and P content of the blood serum was determined and a histological examination of a leg bone of pigs 1527 and 1532 was also undertaken. Table VII shows that pigs 1521, 1527 and 1532 had a lowered blood Ca as compared with pigs on a normal diet. This observation, combined with the results of the pathological study described below, strongly suggested that the symptoms were rachitic in nature.

Pathology. It was at first thought that the symptoms might be due to an infective arthritis, although there were no similar symptoms in any of the other animals in the piggery. A bacteriological and histological examination was carried out on all four pigs of the first block. The right foreleg, just above the carpus, was removed at slaughter from the carcase and examined at the laboratory. The joints were examined culturally and the bones dissected. Material selected for histological examination was decalcified in nitric acid, and paraffin sections were cut and stained with haematoxylin and eosin and with haematoxylin and picrofuchsin.

Pig 1447, which showed severe symptoms during life, exhibited marked thickening of the periarticular connective tissue of the carpus and a slight excess of synovial fluid. No bacteria were seen in films and aerobic and anaerobic cultures were negative. The articular surfaces of all the lower limb bones were normal and sawing through the bones revealed no macroscopic abnormality. No macroscopic changes were found in the other pigs.

Histological sections of the lower end of the radius of pig 1447 showed an epiphyseal cartilage which was thicker than normal, varying from 720 to 1,300 microns, and the cartilage cells were irregular. There was some fibrosis of the marrow of the metaphysis and also some rarefaction of the shaft of the bone. Pig 1444, which was the control animal on meat meal, showed a more regular epiphyseal cartilage varying from 360 to 600 microns in width and the bone marrow was cellular throughout the section.

A similar examination was carried out when the second block of pigs was slaughtered. No bacteria were isolated and no macroscopic abnormalities were detected in any of the bones or articular cartilages. Sections of the lower end of the radius of pig 1468, which showed symptoms during life, showed a rather irregular epiphyseal cartilage with small islets of hyaline cartilage some distance on the diaphyseal side of the line and surrounded by a fibrocellular tissue in which were some osteoclasts.

Up to this time we had been unable to account for the symptoms shown and had not found any lesions in the lower part of the limb which could explain the lameness. The histological features, however, suggested a mild degree of rickets. Only the lower part of the limb was taken from the carcase in order to waste as little food as possible, but it was now decided to carry out a more extensive examination on succeeding cases.

The findings of the pathological examination of three of the four pigs kept for a post-experimental period on 8 per cent., increasing to 20 per cent. yeast diet, were as follows :—

Pig 1536. Lame, very weak, poor condition. The elbow joints showed an excess of synovial fluid which was more viscid than normal and turbid. The articular surfaces of the condyles of the humerus showed folding and grooving (Fig. 1) and a longitudinal section through the bone showed a very vascular metaphysis and an irregular epiphyseal line. The bone immediately beneath the articular cartilages was very vascular and soft and blood oozed from the surface. The upper limb bones were all abnormally soft but the other limb bones appeared to be normal. The gastric mucosa showed marked congestion in the fundic area and there was an ulcer 1·5 cm. in diameter. Histological sections of the upper end of the radius showed great thickening of the articular cartilage (up to 2,400 microns) and extensive rarefaction of the underlying bone. The folding of the articular cartilages appeared to be due to this bone destruction and apparently resulted from pressure ; the increased thickness of the cartilage was presumably compensatory. The bone marrow was almost entirely fibrous. The bone just beneath the articular cartilage showed foci of haemorrhage and bone destruction. Throughout the sections there was an excess of osteoid tissue, both as pure osteoid trabeculae and as wide margins to the bone trabeculae. Pronounced osteoporosis was also in evidence.

Pig 1532 was killed at the abattoir and the left fore limb and the internal organs were examined at the laboratory. The stomach showed intense congestion of the fundic mucosa. The elbow joint contained 8 ml. of fluid ; the joint capsule and periarticular tissue were thickened. The articular surfaces showed thin and thick areas and some grooving. The lower limb

bones and joints were macroscopically normal. The articular cartilage of the lower end of the humerus varied from 800 to 2,800 microns in thickness. The epiphyseal cartilage showed several projections up to 1,800 microns long into the underlying metaphysis; the rest of the cartilage was about 500 microns in thickness. The subarticular bone had been largely removed and replaced by a fibrocellular tissue with osteoid trabeculae and foci of haemorrhage. Bony trabeculae were surrounded by thick osteoid zones. The upper end of the radius and ulna showed similar changes.

Pig 1527 was killed at the abattoir and examined as in the case of the previous pig. The gastric mucosa was congested. No bacteria were isolated from the joints of the fore limb. The condyles of the femur showed grooving and puckering of the articular cartilages. The other bones were macroscopically normal. Histological sections of the upper end of the tibia showed no striking abnormality. The lower end of the femur showed marked rarefaction of the metaphyseal zone with replacement by fibrous tissue and the presence of numerous thin walled blood vessels, large numbers of osteoclasts and an area of haemorrhage. The articular cartilage was thickened, up to 2,000 microns. The underlying bone was largely replaced by fibrocellular and osteoid tissue, with many osteoclasts and small islets of cartilage cells (Fig. 2).

It now became clear that the lesions were typical of rickets in the pig as described and illustrated by many authors, notably by Marek and Wellmann (1931), whose description is exactly similar to that given above.

The transfer of four pigs from an 8 per cent. yeast diet to one with 20 per cent. yeast soon produced complete inappetence, although the animals fought for food when offered the ordinary meal mixture in which the protein supplement was meat meal. It is not possible to say whether the bone and joint lesions in these pigs were more advanced than in the animals receiving 8 per cent. yeast only because, in the latter, only the lower part of the limbs was examined. It is recognised in the literature—and our subsequent experience confirmed the view—that the lower limb bones and joints may appear normal even when severe rachitic changes are present in the upper part of the limbs.

Test on Rats. In the course of the experiment the yeast diets were also fed to rats. Nine pairs of rats were given respectively the 8 per cent. and the 20 per cent. yeast diet for a period of 20 weeks. None of these animals showed symptoms of rickets at any time. Macroscopic and histological examination of the bones showed no abnormality as compared with bones of rats kept on the normal stock diet. The blood Ca and the bone ash were determined and were normal, with no significant difference between the two groups. Both diets were readily consumed. It is well known that rats are very resistant to rickets unless the Ca : P balance is drastically upset and it was not surprising that they did not react to the diet in the same way as the pigs. On the other hand, their continued good health confirmed the view that the symptoms shown by the pigs were not attributable to any toxic properties of the diet.

EXPERIMENT 2

This was a small pilot experiment undertaken in order (1) to reproduce the abnormal symptoms which occurred on the 8 per cent.

yeast diet in experiment 1 ; (2) to try to hasten the appearance of symptoms by increasing the proportion of yeast in the diet to 20 per cent. ; (3) to find out whether the symptoms could be prevented by the administration of cod-liver oil, a rich source of vitamin D.

One litter of ten-weeks-old weaners was divided into four groups (3, 3, 2 and 2 pigs in a group). The pigs were group fed. Two meal mixtures were used and each was fed with or without cod-liver oil, of which 1 per cent. was given. The composition of the diets is shown in Table X. It will be noted that a little extra limestone was added to improve the Ca : P ratio in the 20 per cent. yeast diet.

The animals were gradually changed from the normal diet to the experimental ones. The experiment lasted only ten weeks as at that time a serious infection broke out in the piggery and all the pigs had to be disposed of. Although the cause of the infection has never been established, there is no reason to suggest that it was in any way connected with the yeast experiment.

The six pigs receiving the 8 per cent. yeast diet with or without cod-liver oil (groups 1 and 2) grew normally and showed no abnormal symptoms. On the other hand the pigs receiving the 20 per cent. yeast diet did very poorly. After four weeks pig No. 1664, receiving 20 per cent. yeast diet without cod-liver oil, died suddenly during the night. During the afternoon preceding its death it appeared slightly unsteady on its legs but no other symptoms were observed. It had, however, previously had several " fits," probably attacks of tetany. At autopsy the lungs were distended and filled with froth. The stomach showed an acute gastritis in the fundic area. Sections of the lungs showed haemorrhage and oedema. As the animal had been on the 20 per cent. yeast diet for only four weeks the skeletal system was not examined.

On the day following the death of this pig its group mate, pig 1671, suddenly developed severe convulsive " fits " and was not expected to survive. It was, however, still alive the next morning, although in a very poor state. Surprisingly, its condition improved during the day and on the following day it appeared quite normal. For a month the pig behaved normally but grew at a slightly subnormal rate. It then began to leave food and had another fit. On the 56th day of the experimental period the pig was unable to get up in the morning. When forced to move it walked on its knees, the procedure being evidently painful. It was also unsteady on its hind legs. At this stage the pig was slaughtered owing to the outbreak of the infection in the piggery.

The left fore and left hind limbs were examined. The shoulder joint showed a considerable excess of fluid containing shreds of fibrin and the synovial membrane was thickened and hyperaemic. The articular cartilages were very thin but there were no actual defects. The elbow joint showed similar changes and there were deep grooves and fissures in the articular cartilages of the upper ends of the radius and ulna. The hip joint contained blood, due apparently to tearing of ligaments, and a turbid fluid with shreds of

fibrin; the joint capsule was thickened. The articular cartilages were very thin but there were no actual defects. The lower limb joints were all normal. The epiphyseal cartilage of the upper end of the radius varied in width from 900 to 3,000 microns. The metaphysis showed foci of haemorrhage and considerable osteoporosis, with numerous osteoclasts and much osteoid. The bone trabeculae were lined by osteoid tissue up to 50 microns wide and some were composed entirely of it. The marrow was largely fibrous in the immediate vicinity of the metaphysis. The lower end of the femur and upper end of the tibia showed similar changes.

The two pigs receiving the 20 per cent. yeast diet with cod-liver oil did well during the first seven weeks. On the 48th day pig No. 1668 was seen for a short time to be struggling for breath and showed a tetanic spasm. Subsequently it refused part of its food but otherwise appeared normal. Five days later it was found dead in its pen. It had shown no signs of lameness. Autopsy showed a slight excess of clear fluid in the peritoneal cavity and considerable excess in the pericardial sac. The heart muscle was pale and there were a number of subepicardial ecchymoses. The bronchi were filled with pink froth and the lungs were congested and oedematous. The fundus of the stomach was congested. The right fore and hind limbs were examined in the laboratory. The right elbow joint showed a small pit in the articular cartilage covering the radius and grooves and pits were seen on the articular surface of the tibia. The articular cartilages were very thin and easily detached from the underlying bone which was abnormally soft and vascular. The histological changes were exactly as described for the previous pig.

At the time of the death of pig 1668 its group mate, pig 1669, started to refuse food and showed symptoms of stiffness in the legs. It could only move slowly and with difficulty. It had to be killed, owing to the outbreak of infection referred to above, at the same time as pig 1671. Joint changes similar to those in pig 1671 were found, but they were less marked. Cultures of the fluid within the joint cavities yielded no growth. The histological changes were also similar but not so advanced.

Discussion. It is tentatively suggested that the two pigs on the 20 per cent. yeast diet which showed " fits " and died and a third pig which had a fit but recovered, were suffering from calcium tetany due to lowered blood Ca. Such " fits " have been described by several workers and it is unfortunate that we did not at this stage make blood calcium estimations. It is of interest that pig 1669, which had shown lameness and was given cod-liver oil, showed lesions similar to those in the animals which had no cod-liver oil. This suggests that the addition of cod-liver oil was insufficient to counteract the abnormal diet. Reference to the results of the third experiment shows that in no other pig receiving cod-liver oil were symptoms of lameness or rachitic changes observed in the bones, although they were found in varying degrees in animals fed diets of 8 per cent. and 20 per cent. yeast without cod-liver oil.

There is no doubt that the pathological changes observed were those of rickets, perhaps associated with some degree of osteodystrophia fibrosa. There is abundant confirmation of this in the literature—from the paper by Kernkamp (1925) to the recent articles

by Senior (1940), and especially in the comprehensive monograph of Marek and Wellmann (1931).

The results of this experiment showed that the 20 per cent. yeast diet produced severe adverse symptoms early in the experimental period, the appearance of which was slightly postponed by addition of cod-liver oil.

EXPERIMENT 3

In view of the previous findings an individual feeding experiment with 36 pigs was carried out in which an attempt was made to control the factors connected with the development of rickets. The method of experimentation was the same as described in Experiment 1.

Six different diets were used, *i.e.*, six pigs in each block and six blocks in operation. The pigs were Large White weaners purchased immediately after weaning when eight weeks old from the Agricultural Research Council Field Station at Compton and put on the experiment when ten to eleven weeks old. The interval of two to three weeks was allowed to accustom the pigs to the new conditions. The pigs were reared prior to weaning under conditions similar to those at Shinfield. The experiment commenced on August 7th, 1941, and was completed on March 31st, 1942, and the experimental period for each block was 22 weeks (154 days).

The six experimental diets were as follows: (1) 8 per cent. yeast diet (diet A). (2) 8 per cent. yeast diet (diet A), with cod-liver oil. (3) 20 per cent. yeast diet (diet B). (4) 20 per cent. yeast diet (diet B), with cod-liver oil. (5) 8 per cent. yeast diet, with Ca and P adjusted to bring the content of these minerals to the level present in the 20 per cent. yeast diet (diet C). (6) 20 per cent. yeast diet with extra calcium added (diet D). The yeast used was ordinary brewers' yeast.

The composition of the experimental diets used is given in Table XI. The chemical composition of the experimental meal mixtures is given in Table XII, in which the content of Ca and P as well as the Ca : P ratio are also included. It should be noted that in this experiment calcium carbonate B.P. with 38·5 per cent. Ca was used instead of the previously used limestone which contained only 26·5 per cent. Ca. The meal mixtures themselves were also analysed for Ca and P, four samples being taken of each. The actual and calculated figures showed close agreement and the variation within each of the four samples was very small.

A few weeks before the end of the experiment the supply of barley meal ran out, and as none could be obtained locally was replaced by a mixture of 75 per cent. ground oats and 25 per cent. weatings. At the same time the stock of yeast was also running short and it was decided to introduce a new consignment to coincide with the other changes. Only slight variations in the chemical composition of the diets were caused by the changes, and these are shown in Table XI (mixtures A^1, B^1, C^1 and D^1). The change to the new meal mixtures took place a few days before the fourth block of pigs was sent to slaughter, and so it could affect only the last two blocks.

Cod-liver oil was added to diets 2 and 4 at the rate of about 1 per cent. of the diet at the beginning of the experimental period, *i.e.*, about 11·5 g. per day. This amount was kept constant throughout so that at the end of the experimental period the cod-liver oil was added at the rate of $\frac{1}{3}$ per

cent. It was placed once daily directly in the troughs of the pigs concerned. The batch of cod-liver oil used was stated by the manufacturers to contain 1,080 I.U./g. of vitamin A and 100 I.U./g. of vitamin D.

Results. *Growth and Health.* Table XIII gives details of the growth of all the pigs. Fig. 3 shows the average growth curve for each of the six treatments. All the pigs receiving a diet with 8 per cent. yeast (diets 1, 2 and 5) made satisfactory progress. The addition of cod-liver oil to the diet had a statistically significant beneficial effect on the growth of the animals. On the other hand the addition to the 8 per cent. yeast diet of Ca and P to bring their content to the levels present in the 20 per cent. yeast diet was without effect. The pigs receiving this diet grew well except pig 2804, in block 5, which developed an umbilical hernia half-way through the experimental period. Even including this pig in the statistical analysis, no significant difference in growth of the pigs on diets 1 and 5 could be established.

It should be noted at this point that a few weeks before the end of the experimental period a fight took place between the pigs in block 2, and pigs 2766 and 2759 on treatments 1 and 6 respectively were injured and bled considerably; they had to be separated from the other pigs in the block. The pig on treatment 1 soon recovered, but pig 2759 died from a streptococcal infection with secondary acute endocarditis and metastatic lesions in other organs.

Four pigs (Nos. 2723, 2758, 2773 and 2780) receiving the 20 per cent. yeast diet (diet 3) developed abnormal symptoms similar to those described in Experiment 1. The symptoms appeared much later in the experiment that in the pigs on a similar diet but with limestone instead of calcium carbonate as a source of Ca (cf. Experiment 2). The effects of the diet were most marked in the first four blocks and were only very slight in the other two; this may have been due to the change of diet necessitated by shortage of the original foods and also to individual variation between different litters. Various workers have referred to storage of vitamin D in pigs during the suckling period when the animals are given access to sunlight; as the pigs in Experiment 3 were kept under such conditions prior to being brought to Shinfield it is probable that they started the experiment with considerable but varying stores of the vitamin and thus reacted differently to feeding of yeast.

Fig. 4 gives the mean weekly gains in weight of six pigs receiving the 20 per cent. yeast diet and of five pigs receiving the same diet plus cod-liver oil. It will be seen that as judged by the up-and-down fluctuations of the first curve the 20 per cent. yeast diet did not agree with the pigs. Even the two pigs in blocks 5 and 6 which did not develop acute symptoms showed irregular gains in weight. In contrast to this the pigs receiving cod-liver oil (treatment 4) showed smooth growth curves. No animal on this diet had any abnormal symptoms. The addition of extra calcium to the 20 per cent. yeast diet (diet 6) did not improve it to any extent. Here again the

pigs in the first four blocks (Nos. 2732, 2759, 2767 and 2782) developed the acute symptoms with different degrees of severity. In general the behaviour of the pigs on this treatment was similar to the behaviour of those on treatment 3.

It will be noticed that there is apparent disagreement in the results of this experiment with the findings of the previous experiment in which abnormal symptoms appeared on an 8 per cent. yeast diet late in the experimental period (Experiment 1) and much earlier on the 20 per cent. yeast diet (Experiment 2). A possible explanation is that in the present experiment calcium carbonate B.P. (38·5 per cent. Ca) was used as the additional source of Ca, while previously limestone (26·5 per cent. Ca) was employed.

Food Consumption. Table XIV gives details of the amount of food consumed and refused by the pigs on the different treatments. It appears from these figures that the food consumption of the animals receiving the diets containing 8 per cent. yeast was satisfactory ; the differences between diets 1 and 2 and 1 and 5 were not statistically significant. On the other hand, the pigs receiving 20 per cent. yeast without addition of cod-liver oil (diet 3) did not utilise the food as efficiently as the others. Almost from the beginning of the experimental period some of them refused to consume all the meal allotted to them, and food refusals increased as the experiment progressed. Pigs on diet 6 (20 per cent. yeast plus extra Ca) showed a similar picture but the refusals of food were on a rather smaller scale. On both these diets (diets 3 and 6) the pigs showing the acute symptoms had also a poor appetite and on some occasions the daily refusals amounted to 90 per cent. of the food offered. The number of refusals was correlated with the abnormal symptoms.

The addition of cod-liver oil had a marked influence also on food utilisation, and although the number of refusals was rather high on diet 4 (20 per cent. yeast plus cod-liver oil) the quantity refused was small. These refusals mostly took place at the beginning of the experiment and it would seem as if the diet containing 20 per cent. yeast was not very palatable, but that any dislike the pigs may have had for it was promptly overcome in the presence of cod-liver oil.

Ca and P in Blood. Blood samples were taken from the ear at the beginning of the experimental period and after 7 and 14 weeks, and at slaughter a fourth sample was taken from the throat. Table XV gives the results of the estimation of serum Ca and inorganic P. The variation in Ca content in the initial samples is smaller within litters than between litters. The lowest Ca figure recorded at the initial sampling was 9·07 mg./100 ml. The second sample, taken after seven weeks, did not show any marked change in relation to the diet. At the third sampling there was a definite drop in the blood Ca in all the pigs on treatment 3 (20 per cent. yeast) and in some pigs on treatment 6 (20 per cent. yeast plus extra Ca). This is the more noticeable as most of the other pigs showed an increase in Ca over the previous sampling.

The final sampling showed that the abnormal symptoms were associated with low blood Ca. All the pigs receiving the 20 per cent. yeast diet showed a low Ca except those receiving cod-liver oil. Abnormal leg symptoms were seen in all animals on this diet in which the Ca dropped below 8·5 mg./100 ml. The figures for P are variable and the connection with the diet is much less evident though there is an indication that with low Ca the P tends to be low. Although the mean value for blood P for the pigs given extra P is higher than for pigs which had the 8 per cent. yeast diet unaltered the difference is of no statistical significance.

Ash Content of the Bones.—From each of the experimental pigs the fifth external metacarpal was dissected from the right fore leg and the ash content determined (Table XV). Taking the mean figures for each treatment, it appears that the pigs receiving 20 per cent. yeast without cod-liver oil had the lowest ash content. The differences are, however, small and not statistically significant.

Vitamin A in Livers.—All the livers of the experimental pigs were analysed for vitamin A with the results shown in Table XV. All the pigs had some vitamin A in their livers, and the figures, when compared with those previously reported by Foot *et al.* (1938), using the factor 1 Moore Blue Unit = 0·39 I.U., show that the vitamin A supply of the pigs was satisfactory. The mean figures for each treatment are probably more representative than the individual data.

All the pigs receiving cod-liver oil had a very high vitamin A reserve. An interesting point is that the composition of the diet apparently influenced the assimilation of vitamin A, as all the pigs receiving 8 per cent. yeast with cod-liver oil (diet 2) showed a higher storage in the livers than the pigs on the 20 per cent. yeast diet with the same quantity of cod-liver oil (diet 4), the difference being statistically significant. The same difference, also statistically significant, is apparent from a comparison of pigs receiving the yeast diets without cod-liver oil (diets 1 and 3 and 1 and 6) though here the lower food intake of some of the pigs receiving the 20 per cent. yeast diet may complicate the issue.

Pathology.—As a routine at slaughter the right fore limb was removed from each dressed carcase about 2 inches above the elbow joint. The limb was dissected, the condition of the joint noted and the bones examined macroscopically. Radiographs were taken of the lower end of the humerus and the entire radius and ulna after dissecting away the muscles. After radiography the bones were sawn in half and examined and suitable pieces, including the articular and epiphyseal cartilages, were taken from the upper end of the radius and lower end of the humerus for histological study.

Pig 2759 which, as mentioned earlier, died following a fight, showed at autopsy acute bacterial endocarditis with large crumbling vegetations on the mitral valve and septic infarcts in the kidneys. Haemolytic streptococci were isolated in pure culture from the heart valve lesions and from

the spleen and the source of infection was presumably the wounds inflicted during the fight 14 days previously.

Table XVI gives a summary of the macroscopic and histological features of the bones and the X-ray findings. The effects of the six experimental treatments have already been discussed and only a brief account of the pathological changes need be given here. The most severe rickets was seen in pigs on diets 3 and 6, *i.e.*, diets with 20 per cent. yeast and 20 per cent. yeast with added calcium. The rachitic effect of the high yeast diet was not significantly affected by the addition of extra Ca to the diet. On the other hand, addition of cod-liver oil to the diet of animals receiving 20 per cent. yeast produced the best group of pigs.

Some of the animals receiving 8 per cent. yeast showed very slight rachitic changes and there was no significant difference when cod-liver oil was added. Addition of Ca and P to an 8 per cent. yeast diet to bring the content of these minerals to the level present in the 20 per cent. yeast diet failed to produce rickets.

Block 1.—Pig 2723, fed on a diet of 20 per cent. yeast, showed severe puckering and grooving of the articular cartilages of the elbow joint, excess of synovial fluid and thickening of the periarticular tissues, and very soft bones. Sections showed all the features of rickets referred to earlier (Figs. 5 and 6). This pig showed a degree of rickets more marked than any other pig in Experiment 3. The next most severely affected animal was pig 2732, receiving 20 per cent. yeast and extra Ca. The two animals receiving cod-liver oil were normal. It was quite clear from this block that 20 per cent. yeast produced severe rickets, completely prevented by cod-liver oil. Addition of Ca to the 20 per cent. yeast diet seemed to give only partial protection. This was borne out by subsequent blocks.

Block 2.—The results were similar to those of block 1 but less severe changes were present. Pig 2759, which, as already mentioned, died following a fight, showed marked rachitic changes and in addition there was a streptococcal osteomyelitis which made it difficult to assess the significance of the bone changes due to the diet.

Block 3.—Pigs 2773 and 2767, receiving diets with 20 per cent. yeast without and with addition of Ca respectively, both showed marked rickets of about equal severity (Fig. 7.). Both pigs receiving cod-liver oil showed normal bones (Fig. 8), and the animals on 8 per cent. yeast showed only very slight changes.

Block 4.—The macroscopic changes were not marked in any of the pigs in this group ; slight grooves were seen on the condyles of the humerus in the pigs receiving 20 per cent. yeast and the same diet with addition of Ca. Sections confirmed that these two pigs showed mild rickets, all the other pigs being normal. The bone trabeculae were thicker in the two pigs receiving cod-liver oil.

Block 5.—There were no significant differences detectable on macroscopic examination of the bones and almost no rachitic changes in sections.

Block 6.—There was evidence of very mild rickets in the pigs receiving 20 per cent. yeast with and without extra Ca. The other animals showed normal bones.

Radiographs. Radiography was adopted because it was thought that it would give a general idea of the degree of calcification of the bones and, in subsequent experiments, enable us to follow the course of treatment of pigs made rachitic by a high yeast diet. In

an experiment still in progress pigs showing severe symptoms of rickets on a 20 per cent. yeast diet were radiographed and then given cod-liver oil and sunlight over a period of several weeks; the rapid improvement in condition, gain in body weight and better calcification of the bones at subsequent X-ray were very striking.

The pigs in Experiment 3 were eight months old when killed and the bones X-rayed, and it is important to note the times at which the epiphyses unite in normal pigs. Sisson (1938) gives the following times: Lower end of humerus, one year. Upper end of radius, one year. Lower end of radius, three-and-a-half years. Ulna, both extremities, three to three-and-a-half years. None of the pigs examined showed complete unity of the epiphyses. The lower end of the ulna was usually of greatest value in assessing the degree of rickets, the features noted being the width of the epiphyseal cartilage, degree of calcification and regularity of the metaphysis, thickness of the compacta of the shaft and general condition of calcification.

We are much indebted to Dr. Campbell Golding, of the Royal Cancer Hospital, London, for valued assistance in the interpretation of the radiographs. In his opinion the bones show rachitic changes of varying degree and exhibit all the features of rickets.

Table XVI summarises the X-ray findings and the macroscopic and histological changes and gives an idea of the relative severity of the changes in the bones. Typical radiographs are shown in Fig. 9.

REVIEW OF LITERATURE DEALING WITH RICKETS IN PIGS

Kernkamp (1925) described a condition in pigs termed "posterior paralysis" which could be prevented by adding calcium carbonate to the diet and which was shown by the use of X-rays to be rachitic in nature. Photographs of the bones and joints show lesions similar to those in spontaneous and experimentally induced rickets in pigs described by Marek and Wellmann (1931) a few years later. The literature published prior to 1930 has been fully discussed in Marek and Wellmann's monograph and will not be further discussed in this paper. Subsequent work will be summarised briefly since it shows the importance of a proper Ca : P ratio and adequate amounts of these minerals as well as the requirements of pigs for vitamin D. Marek and Wellmann's experiments appear to have been complicated by vitamin D deficiency and by the stress laid by the authors on the importance of "E.A." (Erdkali-Alkalizität: $(CaO + MgO) - P_2O_5$, expressed as mg. equivalents). Subsequent workers have denied the importance of "E.A." and have merely considered the Ca : P ratio and the absolute amounts of Ca and P in the diet.

Loeffel *et al.* (1931) describe rickets in pigs on a diet with Ca 0·211 per cent., P 0·341 per cent., Ca : P 0·62, and they were able to prevent the condition by sunlight or cod-liver oil (1 per cent. of the diet). This study included X-rays, measurement of the breaking strength of the bones, estimation of bone ash and serum Ca and P, and examination of histological sections. These authors state that although the total bone ash diminishes in rachitic pigs the ratio of Ca to P in the ash remains unaltered. They also record that the humerus and femur showed typical rachitic changes whereas the tibia was less severely affected.

Reimer and Smuts (1932) found that rickets produced by a diet deficient in Ca and with optimal P could be prevented by adding 0·5 per cent. cod-liver oil or by feeding limestone.

Spildo (1933), working in Denmark, published an extensive monograph on Ca and P requirements in pigs and stated that, in the absence of sunlight, deficiency of minerals in the diet produced loss of appetite and rickets or osteodystrophia fibrosa. On diets high in P, with a P : Ca ratio of 2·5 or more the animals developed lesions described as osteodystrophia fibrosa and osteoporosis. There was hypocalcaemia, often tetanic spasms, and always marked lameness. On the other hand, animals on a diet low in P with P : Ca about 0·4 developed osteoporosis and slight rickets. They showed no difficulty in getting up and blood P was low and Ca above normal. The level of serum Ca at which tetanic seizures occur is given as 5-6 mg. per cent. Spildo also states that vitamin D helps in utilisation of Ca if the ratio P : Ca is high but is detrimental if the diet is deficient in P.

Bethke *et al.* (1933) used diets of varying Ca and P content and found that the vitamin D requirements can be reduced by using a ration containing optimal amounts of Ca and P. With a Ca : P ratio of 3·62 and P only 0·32 per cent. exposure to sunlight was insufficient to give normal bone ash. The optimal Ca/P was found to be 1·0 to 2·0 and the minimal requirement of P was 0·6 per cent.; under these conditions satisfactory growth and bone formation took place in the absence of vitamin D. (This finding is in agreement with experience at Shinfield (Foot *et al.* (1938), where pigs are ordinarily fattened indoors on normal diets without cod-liver oil.) The experiments of Bethke *et al.* also suggested that a greater reserve of vitamin D was possessed by pigs kept out of doors until weaned during the months of April-June than in animals farrowed in October and put on an experimental diet indoors in December.

Dunlop (1935) studied Ca, P and vitamin D requirements of pigs and concluded that a diet with Ca 0·45 per cent. and a Ca/P ratio of 1/1·3 was optimal for growth and did not require addition of vitamin D. If the Ca is lower than 0·25 per cent. or the P less than 0·35 per cent. no amount of added vitamin D will give normal growth. Bone ash estimations were made using the tibia and the normal ash was considered to be 55 per cent.

Lund (1935), in a study on Ca and P metabolism in young growing pigs, suggests that the best Ca : P ratio is 1·25 to 1·30. If it is much higher the pigs stop growing or become rachitic; if it is much lower osteoporosis and stiffness or tetany develop.

Theiler *et al.* (1937) studied the effects of diets of varied Ca and P content. Low P with an abnormal Ca/P caused rickets with low serum P and reduced bone ash. Addition of P cured the condition. Diets low in Ca and with an abnormal Ca/P ratio produced osteoporosis but not rickets; serum Ca was unaffected. The animals were exposed to sunlight.

Kernkamp (1937) mentions that deficiency of Ca in pigs causes wrinkled and folded articular cartilages and often actual erosion.

Johnson and Palmer (1939, 1941) showed that white pigs are less liable to become rachitic under the same conditions of diet and exposure to sunlight as coloured pigs. White pigs become rachitic more slowly owing to greater storage of vitamin D prior to being put on the experimental diet. The same authors in a further study (1941) stress the importance of vitamin D in the rations of fattening pigs and state that the need for vitamin D was inversely proportional to the Ca and P content of the ration at levels fed in their experiments.

Marek *at al.* (1940) found that rickets could be produced in young white pigs in absence of sunlight by diets deficient in Ca or P or with a great excess of Ca or P or with very high or very low " E.A." They agree that the ratio of Ca to P is of more importance than the absolute amounts and suggest the minimal needs for young pigs at 4·09 g. Ca and 1·85 g. P for each 10 kg. body weight and an " E.A." of plus 25 mg. equivalents. These requirements are in the absence of sunlight and in this respect it was found that Yorkshire pigs were more resistant than coloured Mangalica pigs (the authors were apparently not aware of Johnson and Palmer's work). Arrest and cure of experimentally induced rickets was obtained by a single subcutaneous injection of 50,000 I.U. of irradiated ergosterol and 1,000 I.U. of vitamin A ; better results were obtained by daily doses of 150 I.U. of vitamin D per 10 kg. body weight.

Weakley and Duxtmann (1938) studied the composition of various parts of the skeleton in animals on different diets and found in pigs that diet had very little effect on bone composition and that symptoms of disease long preceded any change in the mineral composition of the bones.

Senior (1940) tried the effects of diets with Ca/P ratios varying from 1·30 to 1·45 on young weaned pigs which had never had access to sunlight nor received cod-liver oil, although the sows were allowed out during pregnancy and after farrowing. Even with Ca/P ratio of 1·4/1 and a high level of Ca and P (Ca 1·77 per cent.) it was found that rachitic symptoms developed and were not prevented by inclusion of vitamin A in the diet, but were readily prevented or cured by sunlight or vitamin D. The author stresses that pigs require to build up prior to the fattening period a considerable store of vitamin D if they are to remain healthy even on a diet with optimal Ca and P but without vitamin D or access to sunlight. The same author produced rickets in groups of pigs on a diet containing 0·78 per cent. P and Ca varying from 0·59 per cent. to 1·19 per cent. (Ca/P 0·76 to 1·53). In a second experiment the sows were given plenty of sunlight during pregnancy and lactation and here too the piglets developed rickets, although the period before symptoms of stiffness and lameness occurred was longer. The latent period was longer in pigs receiving 0·96 per cent. Ca than in those receiving only 0·51 per cent. Ca. When pigs were given cod-liver oil, 1 pint per cwt. of food, and allowed access to sunlight no symptoms of rickets developed with Ca/P ratios varying from 0·5 to 2·0.

Aubel *et al.* (1941) studied the Ca requirement of pigs, using nine-weeks-old animals kept on an experimental diet for 24 weeks and given plenty of sunlight. Using a P content of 0·3 per cent., they found that a Ca content of 0·6 per cent., 0·8 per cent. and 1·0 per cent., all gave good growth and bone formation. A Ca level of 0·25 per cent. was found to be on the border-line when sunlight was available, two of five animals on this diet showing abnormal development and lowered bone ash and breaking strength. Raising the level of Ca above 0·41 per cent. produced no further improvement. The authors mention that diets too low in Ca caused poor growth in the early stages followed by recovery later on, and suggest that young pigs may require more Ca than older animals. Another possible explanation is that since the experimental period lasted from December to May, the amount of sunlight available was much greater towards the end of the experiment and the animals would therefore grow better on diets too low in Ca for good growth when the sunlight was less. The symptoms developing on diets low in Ca were anorexia, unthriftiness and emaciation, unsteady gait, and finally " posterior paralysis."

Though many reports on the feeding of yeast to livestock have been published (Braude, 1942), diligent search of the literature has failed to reveal more than a few vague references to any ill effects following its use. Thus Kroon (1932) mentioned stiffness in pigs, and Bunger *at al.* (1936) found 10 per cent. yeast in the diet of pigs unsatisfactory. It is interesting to note that Lund (1937), commenting on these findings, suggested that the difficulties experienced were due to calcium deficiency in the diet. Finally, Meteljkow (1937) observed paralysis in fowls on a diet containing a large quantity of yeast.

DISCUSSION

The results of our histological and biochemical work in conjunction with the evidence in the literature summarised above leave no doubt that the addition of yeast to the diet in quantities exceeding 8 per cent. may lead to the appearance of rickets sufficiently severe to interfere with fattening of pigs under our conditions. It should be realised, however, that for practical purposes a diet containing more than 8 per cent. yeast would not be contemplated and that the 20 per cent. yeast diet as used by us must be regarded as a purely experimental ration, which for many reasons would never be considered in practice. We thought that inclusion of yeast at this concentration would make it easier to study the adverse effects it sometimes causes and help to find a remedy for them.

We have already stated that with our ordinary fattening rations containing adequate Ca and P and a Ca : P ratio of about 1 : 1 we were able to fatten pigs indoors without any additional supply of vitamin D. It would seem that such pigs when they reached the stage of baconers were probably on the border line of exhausting any reserve of vitamin D they might have carried over from the suckling period and that the addition of yeast was sufficient to cause an earlier breakdown.

The point at once arises as to how yeast exerts this hastening effect on the appearance of rickets. Yeast is a very rich source of P, of which it contains more than 2 per cent., and naturally its inclusion in any diet in replacement of more usual protein concentrates will increase the P content of the diet without a corresponding change in its Ca content. It is very likely that this increase in dietary P brought about by the addition of yeast is even more significant nutritionally than bare analytical figures would suggest. It is now widely recognised that the availability of P varies according to the source and that presence of phytic acid makes P very much less available for most animals. Most of the cereal components of a practical pig ration contain large amounts of phytic acid and in all probability proximate analysis does not give anything like a true picture of the P really available to the pig receiving such a ration. Yeast, on the other hand, does not contain phytic acid and its P is therefore probably much more available to the pig than the P of the rest of the ration. In this way the addition of yeast

to the diet will bring about a marked shift in the ratio of Ca to *available* P, quite likely in view of the findings of numerous observers (see review of literature) to change a diet just capable of preventing rickets into a diet sufficiently imbalanced to produce clinical rickets in fattening pigs.

It is known that P in inorganic form is fully available to animals, and it seemed to us that if yeast exerted its effects by virtue of the high availability of its P the same effects should be produced by the inclusion in the diet of P in inorganic form in quantities corresponding to those supplied by yeast. This supposition was tested by adding to an 8 per cent. yeast diet enough inorganic P and inorganic Ca to bring the levels of these elements to those present in a 20 per cent. yeast diet. The hypothesis was not borne out by experiment, for while the addition of inorganic P may have caused some slight change in the direction of rickets in pigs which received the 8 per cent. yeast diet in Experiment 3, they did not show anything approaching the symptoms exhibited by the pigs receiving 20 per cent. yeast. It thus seems that the effect of yeast must be due to something other than its P content.

Another experimental result also militates against the explanation that rickets observed by us on high yeast diets was due merely to excess of P. If this were so then the addition of extra Ca to the 20 per cent. yeast diet should have restored the balance and prevented rickets. But this was not the case. There is, nevertheless, good evidence to show that the action of yeast is not unconnected with Ca and P levels in the diet. Thus it will be seen that as the change was made from the limestone supplement used in the first and second experiments to the calcium carbonate B.P. supplement used in the third experiment rickets ceased to appear on the 8 per cent. yeast diet. It is naturally quite possible, however, that batch differences in the composition of diet constituents and changes in the susceptibility of the pigs also played a part. With the 20 per cent. yeast diet an addition of 2 per cent. limestone had no effect, and even an addition of 2·5 per cent. calcium carbonate did not prevent the appearance of abnormal symptoms. It is possible, however, that the extra Ca supplied in treatment 6 of the third experiment was not sufficiently large to correct the balance of Ca to available P, and the question remains open whether a still larger dose of Ca would be able to prevent rickets on the high yeast diet. Experiments to clear up this point are in progress.

Thus the way in which yeast produces rickets has not so far been elucidated, and it would be idle to speculate now at length as to the possible mechanism of this effect. We are now carrying out experimental work which we hope will throw more light on this matter. We should like, however, to refer to the peculiar effect of high yeast diets on the vitamin A reserves of the pigs.

The storage of vitamin A in the livers was definitely affected by the composition of the diet, and it seems therefore possible that a general

metabolic effect is superimposed on the phosphorus effect of high yeast addition.

From a practical point of view our findings do not alter to any extent the conclusions reached by many workers regarding the high value of yeast as a protein supplement for pigs. Dried yeast can replace all the protein supplement normally used in pig fattening mixtures and whatever its rachitogenic properties these can be readily counteracted by the addition of a small dose of cod-liver oil, doubtless by virtue of its vitamin D content. It is quite possible that other sources of vitamin D would do equally well, and there is every reason to believe that pigs could be given any practical amount of dried yeast provided they were given access to sunlight, as they usually are in normal farm practice.

SUMMARY

Three experiments with 94 individually fed pigs are reported in which the nutritive value of dried yeast as the sole protein supplement in meal mixtures for fattening pigs was tested. When large doses of yeast were fed abnormal symptoms were encountered. These were identified as rachitic by macroscopical, radiographic and histological examinations, by determination of Ca and P in blood and by estimation of bone ash. These symptoms were prevented by addition of cod-liver oil and to some extent by supplying additional calcium. The results of these investigations can be summarised as follows :—

1. Dried yeast can supply all the protein required in the diet of fattening pigs. There is no difference in the nutritive value of the three yeast varieties tested, ordinary brewers' yeast, debittered brewers' yeast and fodder yeast.

2. Dried yeast fed at a level of 8 per cent. of the diet with 1·5 per cent. limestone (Ca content 26·5 per cent.) produced rickets in some of the experimental animals. Dried yeast fed at a level of 8 per cent. of the diet with 1·5 per cent. calcium carbonate (Ca content 38·5 per cent.) did not produce abnormal symptoms.

3. Dried yeast fed at a level of 20 per cent. of the diet with an addition of 2 per cent. limestone produced rickets early in the experimental period. Dried yeast fed at a level of 20 per cent. of the diet with 1·8 per cent. calcium carbonate produced symptoms late in the experimental period. Dried yeast fed at a level of 20 per cent. of the diet with 2·5 per cent. calcium carbonate produced symptoms late in the experimental period.

4. Addition of cod-liver oil (declining from 1 per cent. to 0·3 per cent.) to the 20 per cent. yeast diet (cf. under 3) prevented the occurrence of rickets.

5. The adverse symptoms could not be reproduced by adding to the 8 per cent. yeast diet (cf. under 2) Ca and P salts to bring the level of these minerals to that in the 20 per cent. yeast diet (cf. under 4).

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TABLE I

EXPERIMENT 1.—ANALYTICAL DETAILS OF MEAL MIXTURES

		Moisture.	*Crude Protein.*	*Ether Extract.*	*Carbohydrates.*	*Fibre.*	*Ash*
Actual	Weatings	12·1	16·0	3·8	58·7	5·9	3·5
	Barley meal ...	12·2	12·1	2·4	64·8	5·7	2·8
	Flaked maize ...	12·9	10·5	2·5	72·2	1·1	0·8
	Lucerne meal ...	8·5	16·4	2·3	43·0	21·6	8 2
	Meat meal ...	8·0	55·9	15·5	16·1	—	4·5
	Ordinary brewers' yeast	12·2	40·0	0·5	39·1	—	8·2
	Debittered brewers' yeast	10·7	54·0	0·4	26·8	—	8·1
	Fodder yeast ...	9·8	50·8	0·7	31·7	—	7·0
Calculated	Mixture 1 ...	11·8	17·8	4·3	56·9	4·5	4·7
	„ 2 ...	12·2	15·7	2·9	59·6	4·6	5·0
	„ 3 ...	12·1	16·8	2·9	58·6	4·6	5·0
	„ 4 ...	12·0	16·6	2·9	59·0	4·6	4·9

TABLE II

EXPERIMENT 1.—GROWTH OF PIGS AND FOOD CONSUMPTION
(MEAN VALUES FOR PIGS ON THE VARIOUS DIETS)

Diet.	*Initial Weight (lb.)*	*Final Weight. (lb.)*	*Average Daily Gain Per Pig. (lb.)*	*Total Food Consumed. (lb.)*	*Food Consumed per lb. Live Weight Gain. (lb.)*
1. Control*	58	203	1·30	520	3·6
2. Ordinary brewers' yeast*	59	201	1·27	536	3·8
3. Debittered brewers' yeast	58	201	1·27	538	3·8
4. Fodder yeast ...	59	206	1·31	540	3·7

* Seven animals only in these groups as one animal on each diet had to be slaughtered before the end of the experimental period (accidental injury; rectal prolapse).

TABLE III

EXPERIMENT 1.—DEVELOPMENT OF ABNORMAL SYMPTOMS

Pig No. and Sex.	*Block.*	*Diet.*	*No. of Weeks on Experimental Diet when Symptoms first Noticed.*
1451 ♀	1	2	14*
1447 ♀	1	3	14*
1445 ♂	1	4	14
1467 ♀	2	2	13*
1470 ♀	2	3	14
1468 ♀	2	4	14½*
1481 ♀	3	2	14
1480 ♀	3	3	14
1536 ♂	7	3	14*
1527 ♂	8	2	14*
1531 ♀	8	4	14

* Stiff gait and lameness as well as loss of appetite.

TABLE IV

EXPERIMENT 1.—REFUSALS OF FOOD

Diet.	*No. of Occasions on which the Animals Refused Food.*		*Approximate Amount Refused. Lb.*	*Amount of Food Given. Lb.*	*Per cent. Refused.*
	Possible No.	*Actual No.*			
1	784*	80	36·4	3,676·0	0·99
2	784*	142	186·0	3,748·0	4·96
3	896	148	208·1	4,294·0	4·84
4	896	161	152·2	4,318·0	3·52

* Seven pigs only taken into account (cf. footnote Table III).

TABLE V

EXPERIMENT 1.—HEAVY METALS IN YEAST

	Ordinary Brewers' Yeast.	*Debittered Brewers' Yeast.*	*Fodder Yeast.*
	Parts per Million.		
Fe ...	243*	130*	170
Cu ...	62*	12*	15
Mn ...	21	13	17
Pb ...	6·9	6·1	20
As ...	3·5	4·2	1·5

* The striking differences in the figures marked by an asterisk are probably due to the " bottoms " in ordinary brewers' yeast in which the coagulated matter is known to be rich in copper and iron. The debittered brewers' yeast, on the other hand, is prepared from store yeast which contains no " bottoms."

TABLE VI

EXPERIMENT 1.—CALCULATED CALCIUM AND PHOSPHORUS CONTENT OF DIETS

	Per cent. Ca	*Per cent. P*	*Ca : P Ratio.*
Ordinary brewers' yeast	0·21	1·86	
Debittered „ „	0·32	1·82	
Fodder yeast	0·21	1·81	
*Meat meal	0·30	0·30	
Mixed diet with ordinary brewers' yeast ...	0·72	0·64	1·13 : 1
„ „ debittered „ „ ...	0·73	0·63	1·15 : 1
„ „ fodder yeast	0·72	0·63	1·14 : 1
„ „ meat meal (control) ...	0·72	0·51	1·41 : 1

* See note in text, page 164.

TABLE VII

EXPERIMENT 1.—CALCIUM AND PHOSPHORUS CONTENT OF BLOOD SERUM

	Pig No.	*Diet.*	*Age at Slaughter (Days).*	*Inorganic P (mg. per 100 ml. serum).*	*Ca (mg. per 100 ml. serum).*
	1467	Ordinary yeast (showing symptoms)	213	5·20	10·70
	1468	Fodder „ „ „	213	5·44	9·35
	1470	Debittered „ (no symptoms) ...	213	5·40	7·87
Sampled simultaneously	1521	„ „ (late symptoms) ...	271	7·20	8·76
	1527	Ordinary „ „ ...	271	9·80	8·41
	1532	„ „ (no symptoms) ...	264	8·30	9·57
	1564	Normal control diet	242	8·32	12·10
	1590	„ „	225	7·30	11·10
	1592	„ „	225	8·56	10·96

TABLE VIII

EXPERIMENT 1.—VITAMIN B_1 AND MANGANESE CONTENT OF YEAST DIETS

Type of Yeast.	*Mn Content of Diet (pts. per million).*	*Vitamin B_1*		
		In Yeast I.U./g.	*In Daily Ration of 100 lb. Pig (from Yeast only) I.U.*	*In Daily Ration of 200 lb. Pig (from Yeast only) I.U.*
Ordinary brewers' ...	136·3	47·0	6,800	11,100
Debittered brewers'	136·3	33·0	4,800	7,800
Fodder	136·6	10·5	1,500	2,400

TABLE IX

EXPERIMENT 1.—VITAMIN A STORAGE IN THE LIVERS OF THE PIGS

Pig No.	*Days on Experiment.*	*Diet.*	*Symptoms.*	*I.U. Vit. A per g. Liver.*	*Total Vit. A I.U. (hundreds) in the Liver.*
1468	140	Fodder yeast	+	5·1	—
1492	112	Debittered brewers' yeast	—	11·2	217
1494	112	Ordinary „ „	—	15·8	252
1498	112	Fodder yeast	—	19·0	373
1500	112	Control	—	11·9	166
1521	182	Debittered brewers' yeast	+	7·9	153
1527	182	Ordinary „ „	+	14·6	299
1532	168	Ordinary „ „	—	7·9	162
1536	168	Debittered „ „	+	0·9	11
1537	126	Fodder yeast	—	7·3	175

TABLE X

EXPERIMENT 2.—COMPOSITION OF THE DIETS

Weatings	48	40
Barley meal	20	18
Flaked maize	20	18
Artificially dried grass	2	2
Brewers' yeast (ordinary) ...	8	20
Salt	½	½
Limestone (26·5 per cent. Ca) ...	1½	2

TABLE XI

EXPERIMENT 3.—COMPOSITION OF MEAL MIXTURES

Mixture.	*A*	*A^1*	*B*	*B^1*	*C*	*C^1*	*D*	*D^1*
Weatings	48	53	40	45	47	52	40	45
Barley meal	20	—	18	—	20	—	18	—
Ground oats	—	15	—	13	—	15	—	13
Flaked maize	20	20	18	18	20	20	18	18
Dried grass	2	2	2	2	2	2	2	2
Yeast I (ordinary brewers' yeast) ...	8	—	20	—	8	—	20	—
„ II „ „ „ ...	—	8	—	20	—	8	—	20
Calcium carbonate (38·5 per cent. Ca)	1·5	1·5	1·8	1·8	2·0	2·0	2·5	2·5
Sodium chloride	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5
Sodium phosphate (disodium hydrogen phosphate B.P.)	—	—	—	—	2·0	2·0	—	—

TABLE XII

EXPERIMENT 3.—CHEMICAL COMPOSITION OF FEEDING STUFFS AND MEAL MIXTURES

	Moisture.	*Crude Protein.*	*Oil.*	*Soluble Carbohydrates.*	*Crude Fibre.*	*Ash.*	*Ca.*	*P.*	*Ca : P.*
Weatings ...	12·3	16·8	4·8	53·7	8·1	4·3	0·13	0·79	
Barley meal...	11·5	13·6	2·6	63·7	5·6	3·0	0·07	0·41	
Flaked maize	12·3	10·2	3·7	70·0	2·3	1·5	0·12	0·32	
Dried grass...	8·7	15·0	3·3	48·0	11·1	13·9	0·74	0·27	
„ yeast I	6·9	45·9	0·6	36·7	—	9·9	0·22	2·23	
„ „ II	6·8	49·2	0·7	32·8	—	10·5	0·24	2·47	
Ground oats	13·7	11·7	3·2	57·4	11·5	2·5	0·10	0·32	
Mixture A	11·6	16·8	3·7	56·4	5·7	5·8	0·71	0·71	1/1·0
A[1]	12·0	16·9	3·9	54·7	6·7	5·8	0·72	0·73	1/1·01
B	11·1	20·5	3·2	53·8	4·9	6·8	0·94	0·90	1/1·07
B[1]	11·4	21·1	3·4	51·7	5·8	6·9	0·85	0·95	1/1·12
C	11·6	16·7	3·6	55·9	5·6	7·9	0·90	0·93	1/1·03
C[1]	12·0	16·8	3·8	54·1	6·6	7·9	0·91	0·96	1/1·05
D	11·2	20·5	3·2	53·8	4·9	7·4	1·11	0·90	1·23/1
D[1]	11·5	21·1	3·4	51·7	5·8	7·5	1·12	0·95	1.18/1

TABLE XIV

EXPERIMENT 3.—FOOD CONSUMPTION DATA

Diet.	*No. of Pigs.*	*Mean Amount of Food Offered per Pig. Lb.*	*Mean No. of Occasions on which the Animals refused Food (possible 154).*	*Approximate Amount of Food Refused per Pig. Lb.*	*Refusals as Percentage of the Total Amount of Food given.*	*Average Food Consumption per Lb. Live weight Gain (corrected for refusals)*
1	6	750·0	4·2	1·7	0·2	3·97
2	6	761·0	6·0	3·7	0·5	3·72
3	6	724·0	83·0	76·7	10·6	4·26
4	5*	780·0	20·0	12·6	1·6	3·79
5	6	738·0	6·2	4·3	0·6	4·11
6	5	760·0	29·0	34·0	4·5	4·46

* See footnote to Table XIII.

TABLE XIII

EXPERIMENT 3.—GROWTH OF PIGS

Diet.	*Block and Sex.*	*Initial Weight. Lb.*	*Final Weight. Lb.*	*Average Daily Gain. Lb.*
1	1 ♂	58	274	1·40
8 per cent.	2 ♀	54	225	1·11
yeast	3 ♂	47	239	1·25
	4 ♀	56	254	1·29
	5 ♀	45	222	1·15
	6 ♀	41	220	1·16
	Mean	50	239	1·23
2	1 ♂	44	258	1·39
8 per cent.	2 ♂	47	242	1·27
yeast +	3 ♀	43	251	1·35
cod-liver oil	4 ♀	42	241	1·29
	5 ♂	58	261	1·32
	6 ♀	58	258	1·30
	Mean	49	252	1·32
3	1 ♂	60	180	0·77
20 per cent.	2 ♂	37	182	0·94
yeast	3 ♀	40	165	0·81
	4 ♀	59	226	1·08
	5 ♂	53	236	1·19
	6 ♀	49	219	1·10
	Mean	50	201	0·98
4	1 ♂	66	267	1·31
20 per cent.	2 ♀	54	269	1·40
yeast +	3 ♂	43	255	1·38
cod-liver oil	4 ♂	48	240	1·25
	5 ♀	38	230	1·25
	6 *	—	—	—
	Mean	50	252	1·32
5	1 ♀	46	252	1·34
8 per cent.	2 ♂	48	228	1·17
yeast +	3 ♂	58	227	1·10
Ca and P	4 ♂	56	236	1·17
	5 ♀	48	200	0·99
	6 ♀	48	234	1·21
	Mean	51	230	1·16
6	1 ♀	42	209	1·08
20 per cent.	2 ♂	35	died	—
yeast +	3 ♂	40	145	0·68
Ca	4 ♀	57	216	1·03
	5 ♂	62	246	1·19
	6 ♀	54	253	1·29
	Mean	48	214	1·05 (for five)

* In the last litter only 5 pigs were available and treatment 4 was chosen by lot to be excluded.

TABLE XV

EXPERIMENT 3.—SERUM CA AND INORGANIC P, ASH CONTENT OF METACARPAL BONE, AND VITAMIN A IN THE LIVER OF PIGS ON THE SIX DIFFERENT DIETS

Block.	Treatment.	Pig No.	Serum Ca (mg./100 ml.).				Serum P (mg./100 ml.).				Ash Content of Metacarpal Per cent.	Vitamin A in Liver.	
			Initial.	After 7 Weeks.	After 14 Weeks.	Final.	Initial.	After 7 Weeks.	After 14 Weeks.	Final.		I.U./g.	Total · I.U. (hundreds)
1	1	2726	11·1	9·9	10·7	9·6	6·8	6·9	5·7	7·7	51·6	25·0	503
	2	2724	10·5	10·2	12·4	12·6	6·6	7·0	5·9	7·7	53·5	230·0	4,108
	3	2723	11·9	9·4	8·8	8·3	6·1	5·7	5·2	5·8	49·1	6·9	78
	4	2721	11·8	—†	11·2	11·6	6·7	5·8	5·6	7·1	50·4	154·0	3,056
	5	2731	10·7	10·3	11·6	12·6	6·7	6·1	6·3	7·1	53·2	23·5	446
	6	2732	11·8	9·9	11·1	8·1	6·4	6·2	5·7	6·5	50·3	4·0	88
2	1	2766	10·5	10·6	10·6	12·5	6·7	6·0	5·6	7·4	55·5	40·0	669
	2	2757	10·4	10·3	10·9	11·6	6·5	5·3	5·5	7·3	56·0	230·0	3,782
	3	2758	10·9	10·5	8·6	6·7	6·2	5·7	4·8	6·2	50·9	12·9	197
	4	2764	10·7	11·3	11·4	12·9	6·2	5·9	5·9	6·6	57·6	154·0	3,099
	5	2756	11·5	11·3	11·2	11·6	6·5	6·7	5·9	7·4	56·4	8·4	145
	6	2759	11·9	11·1	8·4	Died	6·7	6·0	5·7	Died	Died	7·2	130
3	1	2769	9·2	10·2	10·8	10·9	7·1	6·2	5·8	7·0	51·4	10·2	162
	2	2774	9·1	10·7	11·1	11·0	6·1	6·3	5·7	6·7	52·5	180·0	3,368
	3	2773	9·1	10·8	8·5	6·7	5·8	5·9	5·4	6·2	51·8	7·6	93
	4	2771	9·2	10·2	11·3	10·6	6·0	6·2	5·6	6·8	51·1	142·0	3,020
	5	2770	10·6	10·1	9·7	6·7	6·8	5·7	5·8	6·8	49·9	25·2	414
	6	2767	10·1	10·5	9·1	8·4	6·1	6·5	4·9	5·9	50·6	1·5	15
4	1	2784	12·3	11·3	11·5	12·9	7·1	6·1	7·3	8·0	55·2	13·9	300
	2	2783	12·7	10·2	11·4	11·2	6·5	5·3	7·1	7·0	51·8	224·0	4,255
	3	2780	11·0	11·0	8·9	8·3	6·1	5·7	6·2	7·9	55·8	8·8	177
	4	2775	11·6	11·2	11·7	11·2	6·7	5·8	7·3	7·3	53·0	14·8	3,441
	5	2776	11·6	10·7	10·2	10·7	7·5	6·7	7·2	8·2	53·8	14·8	273
	6	2782	13·1	10·7	10·5	8·5	7·0	5·1	6·7	6·8	53·3	5·7	120
5	1	2798	9·8	11·3	12·4	10·7	5·5	6·1	7·5	7·5	51·9	21·6	404
	2	2795	10·2	11·5	12·7	10·9	6·6	5·9	7·0	6·8	54·9	240·0	4,218
	3	2792	9·4	10·1	10·4	10·1	6·2	5·7	7·0	7·3	48·7	11·0	215
	4	2800	9·6	11·0	10·0	11·1	6·0	5·3	7·1	6·6	55·6	114·0	2,262
	5	2804	9·9	10·4	11·2	10·7	6·1	6·0	7·8	6·2	53·1	27·0	413
	6	2802	10·0	11·3	11·6	—†	6·0	5·3	7·8	7·4	52·4	12·0	261
6	1	2846	10·4	10·7	10·7	11·4	5·6	6·4	6·4	6·8	53·9	19·6	356
	2	2841	11·3	11·5	12·0	11·8	5·4	6·6	7·8	6·2	55·2	184·0	3,756
	3	2840	10·4	10·6	10·9	11·7	5·6	6·4	7·8	6·5	58·0	8·4	181
	4*	—	—	—	—	—	—	—	—	—	—	—	—
	5	2845	—†	10·9	11·1	11·2	5·6	6·5	7·5	6·0	55·8	17·2	302
	6	2844	10·8	11·4	12·2	11·9	5·3	6·4	7·4	6·1	55·8	9·8	200
MEAN	1		10·5	10·8	11·1	11·3	6·5	6·3	6·4	7·4	53·2	21·7	399
	2		10·9	10·7	11 2	11·5	6·3	6·1	6·5	7·0	54·0	201·6	3,914
	3		10·4	10·4	9·3	8·5	5·9	5·9	6·1	6·7	52·4	9·2	157
	4 (5 pigs)		10·6	10·9	11·1	11·2	6·3	5·8	6·3	6·9	53·5	142·4	2,976
	5		10·8	10·6	10·8	10·7	6·5	6·3	6·8	7·0	53·7	19·3	332
	6		11·3	10·8	10·5	8·8	6·2	5·9	6·4	6·6	52·5	6·6	136

* No pig receiving this diet in Block 6. † No figures available.

FIG. 1. Pig 1536, Expt. 1, 8 per cent. yeast diet for 16 weeks, followed by a further experimental period (see text). Lower articular surface of left humerus showing wrinkling and puckering of articular cartilages and thickening of periarticular tissues. × 2/3.

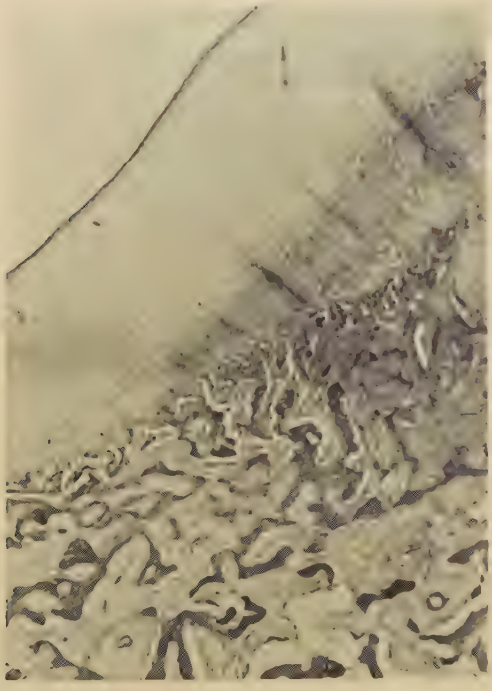

FIG. 2. Pig 1527, Expt. 1, 8 per cent. yeast diet for 16 weeks, followed by a further experimental period (see text). Section through articular surface of external condyle of left femur showing thickening of articular cartilage, extending to a deep tongue at extreme right, rarefaction of sub-articular bone and replacement with a fibrocellular tissue containing osteoid trabeculae, haemorrhage beneath articular cartilage and fibrosis of bone marrow. H. and E. × 18.

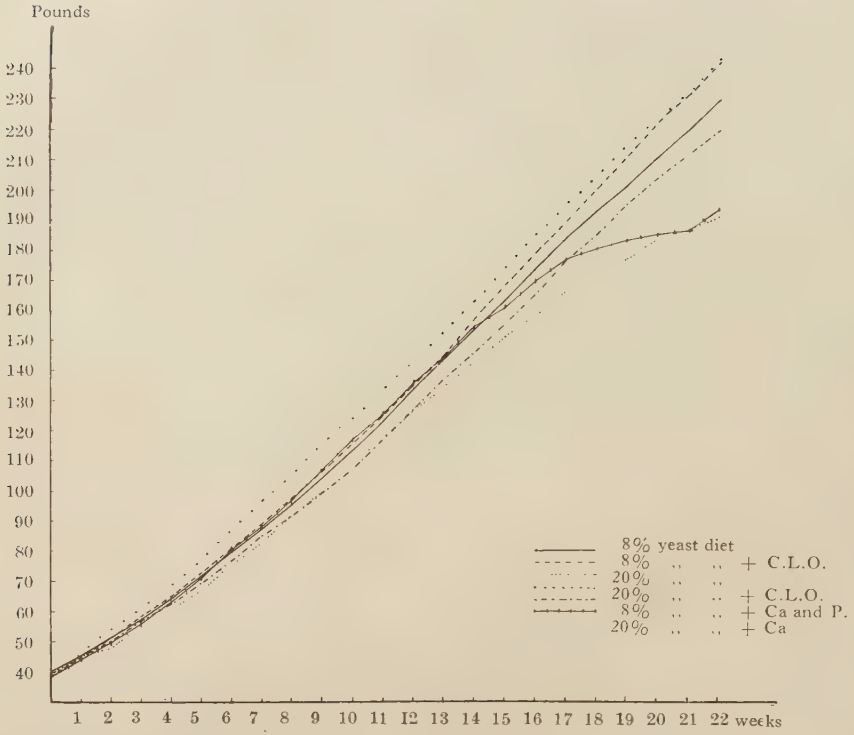

FIG. 3. Growth curves of pigs on each of the six diets used in Expt. 3, each curve representing the average weight of the animals on the different diets.

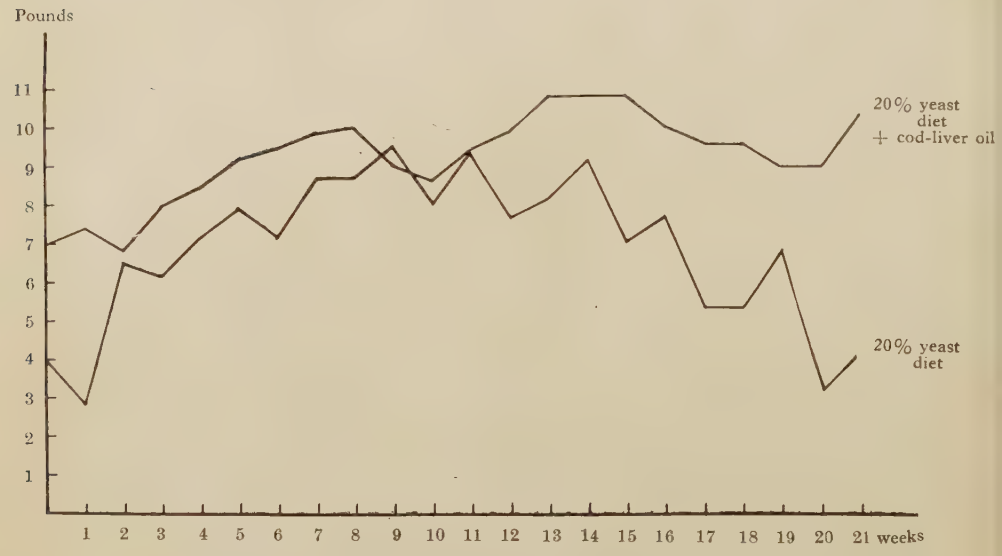

FIG. 4. Graph showing average weekly gain in weight of pigs in Expt. 3 on a diet of 20 per cent. yeast with and without addition of cod-liver oil.

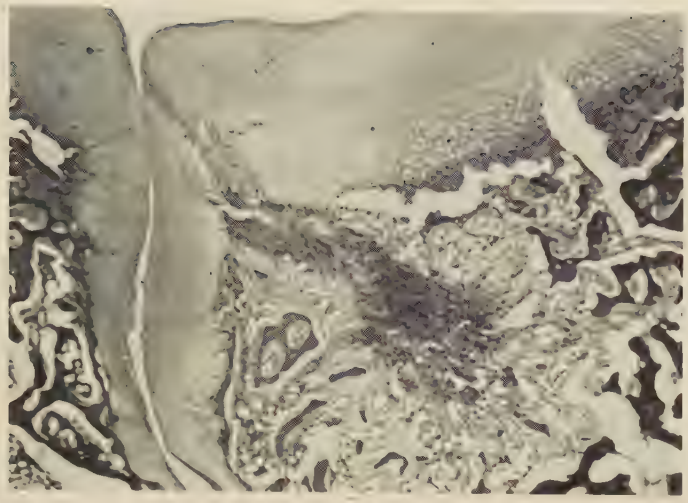

FIG. 5. Pig 2723, Expt. 3, 20 per cent. yeast diet for 22 weeks. Section through articular cartilage of lower end of humerus showing destruction of sub-articular bone with haemorrhage, numerous osteoclasts and formation of a fibrocellular tissue replacing the original bone. Haematoxylin and picrofuchsin. × 18.

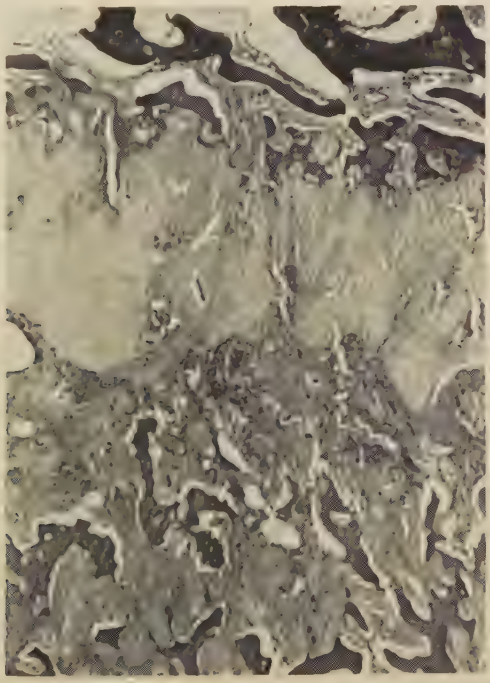

FIG. 6. Pig 2723 (as Fig. 7). Section through epiphyseal cartilage of lower end of humerus. Wavy epiphyseal line with irregular arrangement of cartilage cells: the metaphysis shows numerous small vessels, areas of haemorrhage, numerous osteoclasts and absence of proper ossification. There is marked fibrous replacement of the normal cellular bone marrow. H. and E. × 18.

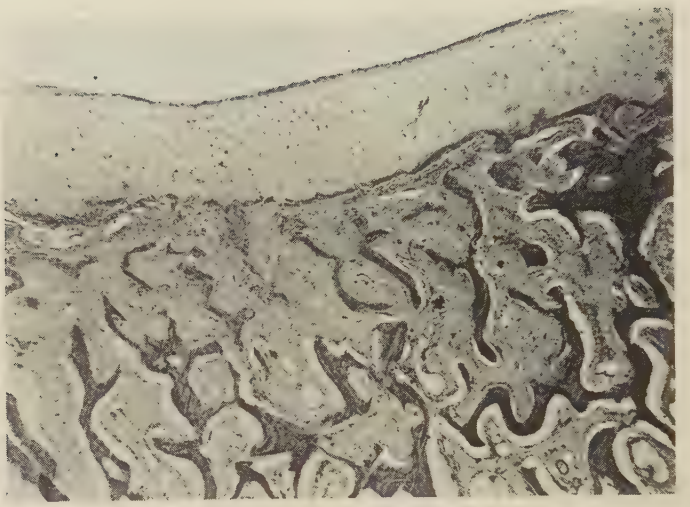

FIG. 7. Pig 2767, Expt. 3, 20 per cent. yeast diet with additional Ca for 22 weeks. Section through upper articular cartilage of radius showing rarefaction of sub-articular bone and replacement by a fibrocellular tissue containing many osteoclasts. The sub-articular bone at the extreme right of the field is more nearly normal. H. and E. × 30.

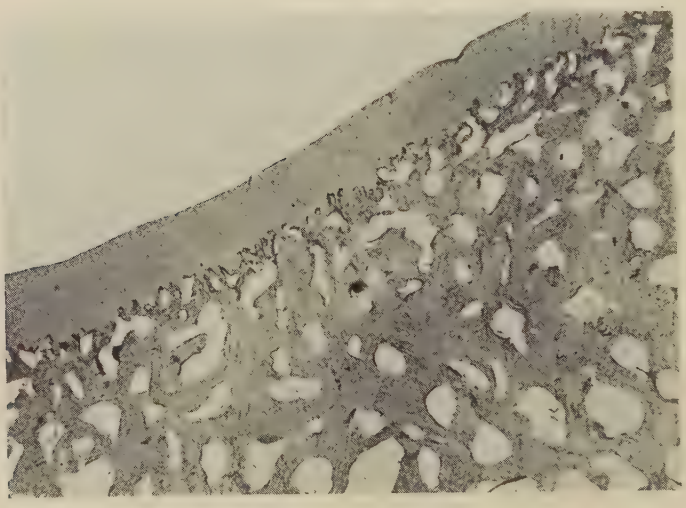

FIG. 8. Pig 2784, Expt. 3, 8 per cent. yeast diet for 22 weeks, no symptoms. Section through upper articular cartilage of radius to compare with Fig. 9. The broad trabeculae of sub-articular bone are in marked contrast to the appearance illustrated in Fig. 7. The cellular bone marrow has fallen out during section cutting. H. and E. × 18.

FIG. 9. Radiographs of the radius and ulna of three pigs in Block 3, Expt. 3. That on the left (Pig 2771, 20 per cent. yeast diet with cod-liver oil) shows a normal well calcified bone. The forearm in the centre (Pig 2773, 20 per cent. yeast diet) is markedly rachitic but shows evidence of healing in the form of Ca deposition in the metaphysis of the lower extremity of the ulna. The bones on the right (Pig 2767, 20 per cent. yeast diet with additional Ca) show severe rickets with marked osteoporosis, widened epiphyseal lines and defective calcification of the metaphyses. All three animals were litter mates and the normal bones are larger and show more compact bone than the others.

TABLE XVI

EXPERIMENT 3.—CONDITION OF BONES OF RIGHT FORE LEG (RADIUS, ULNA AND LOWER END OF HUMERUS) INDICATED BY NUMERALS, INCREASING FROM 1 (BEST CALCIFIED) TO 10 (SEVERE RICKETS)

Block No.	*Pig No.*	*Diet.*	*Radiographs.*	*Macroscopic Features.*	*Histological Features.*	*Total.*
1	2726	1	8	8	4	20
	2724	2	6	2	2	10
	2723	3	10	10	8	28
	2721	4	7	2	2	11
	2731	5	6	4	4	14
	2732	6	8	6	6	20
2	2766	1	4	4	2	10
	2757	2	6	2	2	10
	2758	3	9	8	6	23
	2764	4	7	2	2	11
	2756	5	6	4	4	14
	2759	6	10	8	8	26
3	2769	1	1	1	1	3
	2774	2	3	2	2	7
	2773	3	9	9	9	27
	2771	4	2	3	5	10
	2770	5	7	4	4	15
	2767	6	10	10	10	30
4	2784	1	3	2	2	7
	2783	2	2	1	1	4
	2780	3	8	4	4	16
	2775	4	1	1	1	3
	2776	5	2	3	4	9
	2782	6	7	4	3	14
5	2798	1	4	2	3	9
	2795	2	5	1	1	7
	2792	3	6	2	2	10
	2800	4	2	1	1	4
	2804	5	5	1	3	9
	2802	6	1	3	3	5
6	2846	1	1	2	1	4
	2841	2	5	2	1	8
	2840	3	3	3	2	8
	2845	5	2	2	2	6
	2844	6	4	2	3	9

Assessment of bones of all pigs on each of the diets :—

Diet.	*Total Score.*	*Mean Score per Pig.*
1. 8 per cent. yeast	53	8·83
2. 8 „ „ + C.L.O. ...	46	7·67
3. 20 „ „	112	18·67
4. 20 „ „ + C.L.O. ...	39 (5 pigs only)	7·80
5. 8 „ „ + Ca and P ...	67	11·17
6. 20 „ „ + Ca	104	17·33

Note.—The method of assessment is relative only: the "mean score" for a normal pig would probably range from 3 to about 10.

THE SUCCESSFUL FATTENING OF PIGS ON DIETS CONTAINING 20 PER CENT. OF BREWER'S YEAST

By

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INTRODUCTION

THE value of yeast as a protein concentrate for growing pigs has been demonstrated by many workers (see Braude, 1942) and has recently been confirmed by experiments carried out at this Institute by Macrae, El-Sadr and Sellers (1942). The last-mentioned authors fed 5 to 20 per cent. of a cultivated yeast, *Torula utilis* (49 per cent. crude protein), and concluded that the capacity of the proteins in this yeast to supplement those of maize was the same as that of casein. The animals remained normal throughout the experimental period. However, in some earlier experiments at this Institute (Chick, Macrae, Martin and Martin, 1938), in which a commercial preparation of dried brewer's yeast was added to a synthetic diet in order to furnish the vitamins of the B group, some of the animals manifested symptoms indicating muscular or joint trouble. I am indebted to Sir Charles Martin, F.R.S., for the following summary based on notes made at the time.

The pigs, three in number, were the controls in an experiment designed to determine what water soluble vitamins other than aneurin, riboflavin and nicotinic acid were required by pigs.

Their diet consisted of starch 71, casein (" Glaxo ashless extracted ") 21, cottonseed oil 2·5, cod-liver oil 2·5, salt mixture 3, to which was added 4 per cent. dried brewer's yeast. The experiment was started when the pigs, which were exposed to sunshine, were 45 to 50 lb. in weight. They gained an average of 10·9 lb. weekly and became very fat. After they had been on the diet for four weeks, it was noticed that they were very lazy, lying or sitting most of the time unless stimulated. Laziness increased, and shortly afterwards weakness of the hind legs was observed. They seemed to have difficulty as well as disinclination in rising, suggesting that it was painful to support their weight on their legs. There was no swelling of their joints nor, apparently, pain on handling them.

The laziness and weakness became progressively more marked until by the tenth week they had difficulty in getting on to their legs after lying for some time. The gilt (No. 54) always ate sitting down, and frequently progressed on her knees. The others fed sitting down occasionally. They all ate well and were consuming between them 5·4 kg. of dry food daily. When constrained for examination they seemed strong.

At the eleventh week, when their weights were 155, 145 and 184 lb. respectively, maizemeal was substituted for the starch in their diet, and it was noticed that even after four days they were less apathetic. All were more lively, the gilt could rise unaided, and they all ate standing. The improvement was maintained, and by the fifteenth week only slight laziness and stiffness after rising, which soon passed off, distinguished them from normal pigs.

The cause of the symptoms was not ascertained. That they were nutritional in origin seemed likely, as they disappeared following the substitution of maize for starch, although the animals continued to have the same amount of yeast. The behaviour of the pigs was reminiscent of early rickets in a fat animal, but as they were ingesting 0·72 g. Ca, 0·35 g. P and 2·5 g. cod-liver oil per 100 g. diet, and were exposed to sunlight most of the day, rickets would have been improbable.

Whatever its aetiology, the condition does not appear to resemble any of the forms of stiffness described by Seddon and Fraser (1929), the ataxia described by Wintrobe, Mitchell and Kolb (1938; see also Wintrobe, Miller and Lisco, 1940), or the mostly transient forms of " cramp " and allied porcine complaints which are familiar to farmers and veterinarians, and attributed to a variety of causes (see, *e.g.* Plimmer, 1920). Somewhat similar symptoms in pigs fed on yeast were observed by the workers at Shinfield and are reported elsewhere in this issue (Braude, Kon and White, 1942). The early findings were brought to our notice by Dr. H. D. Kay, and we therefore thought it desirable to determine whether or not yeast was capable of producing toxic symptoms in fattening pigs. In the present experiment dried brewer's yeast was fed to the extent of 20 per cent. of the ration on a dry weight basis.

Dried brewer's yeast is rich in protein, in the B vitamins, and in phosphorus; the batch which was employed in the present experiment was found to contain 7·99 per cent. N (49·9 per cent. protein) and 2·69 per cent. P (none as phytic acid P) on a dry-weight basis. Of these constituents, it would be unlikely that the protein *per se* would prove harmful if fed in large amounts (see Schalk, 1940) while steps would normally be taken to balance the phosphorus by the addition of calcium and by allowing adequate vitamin D.

In order to provide control animals to those receiving 20 per cent. of yeast, a second group of pigs was fattened on a diet containing 16 per cent. of fishmeal and adjusted so as to contain approximately equal amounts of total energy, crude protein, calcium and phosphorus.

EXPERIMENTAL

Twelve Wessex Saddleback pigs, which had been farrowed between April 20th and 25th, 1941, were received from a farmer on July 12th, 1941, and placed on experiment four days later, *i.e.*, at 82 to 87 days of age. Details of the sex and weight of the individual animals, as well as of the composition of the diets, are given in the accompanying tables. The yeast and fishmeal groups were housed in separate pens, and were fed twice daily. Each group received the same total food intake, but individual feeding pens were not employed and resultant figures for ratio of food intake to bodyweight increase should therefore be accepted with some reserve. One of the yeast pigs had to be slaughtered early in the experimental period owing to the development of an abscess at the site of castration, but as the food offered was invariably consumed in the case of each group,

the experimental design was not invalidated. Access to the open air was allowed for about ten hours daily, and there was ample room for exercise.

Both yeast and fishmeal groups grew very well indeed, and in neither case did any of the animals show the slightest evidence of untoward symptoms. At the end of the 14 weeks' experimental period (*i.e.*, on October 22nd, 1941) the yeast group was slaughtered, while—in order to reduce the risk of "taint"—the fishmeal group was gradually transferred to the yeast diet before being slaughtered on November 18th, 1941. At the time of slaughter carcase weights and measurements (Davidson, Hammond, Swain and Wright, 1937; Callow, 1942) were recorded (see accompanying tables). The figures obtained, which have been discussed with Dr. E. H. Callow, indicate that whereas three of the five yeast animals were slightly fatter than expectation, all of the fishmeal controls were within the normal range of back-fat measurements. *Post-mortem* examination revealed animals which were normal in all respects. Appropriate material from the upper limb joints was removed for histological examination, but the sections prepared were found by Mr. D. McFarlane (this Institute) to be perfectly normal. The palatability of the flesh (when consumed as pork) from both groups was excellent and devoid of any trace of "taint" or unwelcome "flavour."

DISCUSSION

There was no evidence of any harmful effect from feeding large quantities of dried brewer's yeast to fattening pigs, although it would obviously be dangerous, as in the case of cereals, not to balance the high phosphorus content of such a diet.

In view of the fact that the plane of nutrition was not varied throughout the fattening period, and that the animals were group fed (with opportunities for scattering the food) the recorded mean gains of 1 lb. bodyweight per 3·97 lb. of yeast diet and per 3·79 lb. of fishmeal diet were very satisfactory. They do not, of course, equal those previously reported for animals on more economically planned yeast diets (Macrae *et al.*, 1942), but, in conjunction with the carcase qualities, they confirm the harmless nature of both foodstuffs. The excellent palatibility of the flesh, and absence from taint, are noteworthy.

In the presence of adequate vitamin D, and with a Ca/P (*not* CaO/P_2O_5) ratio of 1 : 1, the relatively large amounts of calcium and phosphorus (1 per cent. of the diet in each case) permitted of normal skeletal development.

SUMMARY

(1) Wessex Saddleback pigs were successfully fattened on a diet containing 20 per cent. of dried brewer's yeast. The ratio of food consumed to gain in bodyweight for the whole fattening period

was 3·97 : 1 (group-fed basis). Carcase quality was satisfactory, palatability was excellent, and there was no evidence of any injurious effect upon the animals themselves. It would therefore appear that, providing care be taken to balance its high phosphorus content, yeast may with safety be fed in large amounts to young pigs.

(2) Control animals receiving a diet containing 16 per cent. of fishmeal were likewise fattened with good results. After a short " finishing period " on a 20 per cent. yeast diet there was no trace of taint in the carcase. The ratio of food consumed to gain in body-weight (from commencement of experiment to finishing period) was 3·79 : 1, indicating that at these levels there was little difference between the yeast and fishmeal as protein concentrates.

ACKNOWLEDGMENTS

Acknowledgments to Sir Charles Martin, F.R.S., to Dr. E. H. Callow (Low Temperature Research Station) and to Mr. D. McFarlane have been made in the text. The diets were planned in conjunction with Dr. T. F. Macrae (Lister Institute). Dr. J. S. Paterson kindly took charge of the experiment for a time during my absence on other work. Thanks are due also to Mr. A. Tyrell, for his care of the experimental animals, and to Mr. G. Childs for undertaking certain analyses. Helpful information was received from Dr. E. G. White (Royal Veterinary College) and from Mr. R. Braude (National Institute for Research in Dairying).

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TABLE I

DIETS AND WEIGHT GAINS DURING FATTENING PERIOD
(16.7.41-22.10.41)

(*a*) 20 PER CENT. YEAST GROUP

Composition of diet :—

Dried brewer's yeast	20·0
Weatings	40·0
Barley meal	34·5
Ground chalk	2·5
Table salt	0·5
Crude ferric oxide	0·1
Cod-liver oil (added daily)	1·5

Total Ca 1·129 per cent. (as Ca)
Total P 1·080 per cent. (as P)
Digestible crude protein 16·26 per cent.

Weight gains of experimental animals :—

Pig.	*Initial Weight.* (16.7.41)	*Final Weight.* (22.10.41)
7 male	32·6 kg.	78·2 kg.
8 „	31·0 „	—
9 „	29·0 „	99·8 „
10 „	28·2 „	82·4 „
11 female	28·2 „	86·8 „
12 male	24·1 „	72·4 „
	173·1 „ (6 animals)	
	142·1 „ (5 „)	419·6 „ (5 animals)

Total increase (5 animals) = 277·5 kg.
Average individual increase = 55·5 kg.
Total food consumed (5 animals) = 1,102·2 kg.

$$\therefore \text{ ratio } \frac{\text{food consumed}}{\text{gain in bodyweight}} = 3{\cdot}97 : 1$$

(*b*) 16 PER CENT. FISHMEAL GROUP

Composition of diet :—

Fishmeal	16·0
Weatings	40·0
Barley meal	42·0
Table salt	0·5
Crude ferric oxide	0·1
Cod-liver oil (added daily)	1·5

Total Ca 1·118 per cent. (as Ca)
Total P 1·204 per cent. (as P)
Digestible crude protein, 16·63 per cent.

Weight gains of experimental animals :—

Pig.	*Initial Weight.* (16.7.41)	*Final Weight.* (22.10.41)
1 female	34·4 kg.	99·6 kg.
2 male	30·4 „	96·0 „
3 „	29·3 „	87·6 „
4 female	28·8 „	88·4 „
5 male	28·6 „	81·6 „
6 „	21·8 „	68·6 „
	173·3 „	521·8 „

Total increase (6 animals) = 348·5 kg.
Average individual increase = 58·1 kg.
Total food consumed = 1,322·7 kg.

$$\therefore \text{ ratio } \frac{\text{food consumed}}{\text{gain in bodyweight}} = 3.79 : 1$$

TABLE II

CARCASE MEASUREMENTS

Pig.	Hot Carcase Weight (lbs.).	Length (mm.) (a) Body Cavity.	Length (mm.) (b) Leg.	*Back Fat (mm.) A	B	C_3	C_2	C_1	Calculated Value of T. †	Notes.
7 M.	121	730	537	50	21	25	24	35	19·0	Yeast group
9 M.	134	753	568	52	29	40	38	43	21·0	slaughtered at
10 M.	132	732	552	46	26	35	29	38	20·6	termination of
11 F.	145	759	548	44	21	31	26	34	22·4	experiment,
12 M.	113	690	554	40	15	27	17	27	17·8	22.10.41.
1 F.	186	827	623	44	25	35	28	36	28·7	Fishmeal
2 M.	179	819	610	47	26	35	29	35	27·5	group slaugh-
3 M.	163	776	585	48	25	32	22	32	25·1	tered after a
4 F.	168	762	590	46	23	36	28	32	25·8	finishing per-
5 M.	151	774	578	51	26	34	25	32	23·3	iod on yeast
6 M.	130	724	552	47	21	29	25	32	20·3	diet, 18.11.41.

* Letters represent thickness at (A) shoulder, (B) last rib, (C) front, middle and back of " eye muscle."

† Calculated from equation, $T = 1{\cdot}4 + 0{\cdot}149W$,
where T = thickness of back fat (B) in mm.
and W = weight of cold carcase in lb.

The values for W have been derived by subtraction of 3 lb. from weights up to 143 lb., and of 4 lb. from weights of 144-189 lb.

It will be seen that in the case of the yeast group, the calculated values for T are 101 mm. in the aggregate, whereas the actual measurements total 112 mm., *i.e.*, slightly fatter than expectation. In the case of the fishmeal group, the calculated and found values are 151 and 146 mm. respectively. In view of the small numbers of animals employed, and of the possible other variables in the diet, *e.g.*, cereal protein and NaCl [see Moir (1934-35), Vilner *et al.* (1936)] these differences between yeast and fishmeal N may not be significant.

RICKETS IN CALVES

By

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It is generally recognised that rickets of young animals and osteomalacia of adult animals develop on similar lines. Osteomalacia has often been described as adult rickets. Both conditions are characterised by changes associated with defective calcification of the bones. Throughout life bone is being frequently broken up and new bone laid down, and in both rickets and osteomalacia it is in the new osseous tissue that the failure to deposit mineral salts occurs. Rickets differs from osteomalacia in that it affects animals in which the epiphyseal cartilages still persist and there the changes occur which differentiate it from osteomalacia. In these regions, the so called "provisional zones of calcification," deposition of the calcium phosphate is deficient in rickets. In cattle the cartilages of the proximal bones of the extremities persist for two or three years and hence rickets may be noted in these animals up to that age. At these centres the defective deposition of calcium phosphate with the absence of the calcification zone is followed by enlargement from pressure and the irregular production of spongy osteoid tissue. The cartilage is broken into islets surrounded by this new zone. From pressure also the rows of cartilage cells become fanlike in their disposition and the area is very vascular.

In experiments carried out by Theiler these lesions were set up when bovines received inadequate quantities of phosphorus in the food while a calcium supplement containing no phosphorus aggravated the disease. Bran which is rich in phosphorus and poor in calcium proved to be a good preventive.

Rickets and osteomalacia in bovines have been recorded in all parts of the world but are particularly common on phosphorus-deficient pastures. There, rickets was noted particularly in calves which had lost their dams early in life or when they received an inadequate supply of milk where the cows had defective udders.

In this country rickets in calves is only occasionally recorded and this may be due to the fact that adequate quantities of phosphorus in their food is generally provided.

In April, 1939, this affection came under our notice in a batch of three calves in a dairy herd in Cheshire. The disease had not previously been observed in this herd and the calves in another batch at the same time were quite normal. The calves were pail fed but had been given a proprietary food, the composition of which was not ascertained. All the calves were Shorthorn heifers about five or six months old. One of them was in an advanced state of the disease and was so emaciated and weak that it was unable to rise. This calf was destroyed and not forwarded for examination. The other two calves were sent into the Department for investigation.

The symptoms were similar in all cases. Their condition was very poor. The extremities of the bones of the limbs were so greatly thickened that the carpal, tarsal, the metacarpo- and the metatarso-phalangeal and interphalangeal joints appeared to be enlarged. The chondro-costal joints were also thickened. The calves were somewhat stiff in their gait. A prominent feature was the inability to close the mouth and the protrusion of the tongue for two or three inches. The bodies of the mandibles and the rami appeared to be thickened and somewhat soft. As a result there was considerable salivation and the calves had difficulty in feeding and particularly in picking up and grinding the hay which was provided. They could take mash fairly well, and they ruminated normally but slobbered a good deal. Their temperatures were normal.

Samples of the blood of these two calves and another older heifer were kindly analysed for us by Dr. Gaddie, of the Biochemical Department, with the following result given in mg. per 100 c.c. :—

	Plasma P.	*Serum Ca.*	*Serum Mg.*
1. Red heifer	3·48	10·90	3·28
2. White heifer	3·37	8·00	2·51
3. Control	2·82	9·50	2·37

There did not therefore appear to be any significant changes in the phosphorus, calcium or magnesium content of the blood.

The two calves were then subjected to the intradermal tuberculin test and No. 2 gave a pronounced reaction. A diffuse, tender swelling formed around the point of injection and the fold of skin increased in thickness from 3 mm. to 15 at the 48th hour and 16 mm. at the 72nd hour. In calf No. 1 the measurements were 4·5, 6 and 6 mm. respectively.

The reacting calf, No. 2, was then destroyed. On post-mortem examination the only lesions of tuberculosis found consisted of some red patches of broncho-pneumonia. The mucous membrane of the abomasum was studded with small white nodules due to infestation with Ostertagia larvae. The mandible was thickened and soft, and the ends of the bones of the limbs enlarged. The carpal, stifle and tibio-tarsal joints contained some clear synovial fluid and the fringes of the synovial membranes were thickened and reddened.

In some joints such as the shoulder and stifle the cartilages were uneven and wrinkled and some areas denuded of cartilage. The chondro-costal joints were enlarged. The epiphyseal cartilages of the limb bones were irregular and the inner aspect of the periosteum very vascular.

Microscopical examination of sections of the ends of the long bones showed that the line of junction of the cartilage with the developing bone was very wavy. There was no evidence of a calcification zone, the new osteoid tissue was very open and spongy, and calcification was defective. Osteoblast cells were arranged in rows on the surface of the plates. Similar osteoid tissue was a feature also of sections of the mandible. The tissue between the osteoid plates was largely made up of fine fibrous tissue.

The remaining calf (No. 1) was retained and 1 ounce of bone meal was added daily to its food. This calf slowly improved in condition and growth. In three months the thickening of the joints disappeared. The tongue was completely retracted in the mouth and salivation ceased. It fed and ruminated normally. It was slaughtered fat at the end of the year and then presented no abnormalities.

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